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MYOTONIC SYNDROMES: ANALYSIS OF FACTORS WITH PATHOGENETIC AND PROGNOSTIC SIGNIFICANCE

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INTRODUCTION

CLASSIFICATION AND MAIN CLINICAL FEATURES OF MYOTONIC SYNDROMES

Myotonic syndromes (MS) include two main groups of diseases characterized by myotonia and a variable degree of myopathy: c**ongenital myotonias** (CM) and **myotonic dystrophies** (DM) (1-4).

The myotonic phenomenon, which typically distinguishes these pathologies, defined clinically as the difficulty to relax a muscle after maximum voluntary contraction, derives from a dysfunction in voltage-gated ion channels on the skeletal muscle membrane, the sodium channel (Na_v) and chloride channel (CLC), which lead with different mechanisms to altered excitability and repolarization of muscle fibres with the characteristic repetitive discharge on electromyography (EMG). Myotonia can be present in specific regions of the body (eyes, jaw muscles, tongue, hands, or legs) and can also affect smooth muscle, with frequent involvement of the gastrointestinal tract. Repeated exercise or repeated contractions usually improve myotonia: this is known as the warm-up phenomenon.

Congenital myotonias (CM), characterized by autosomal dominant (AD) or recessive (AR) transmission, are extremely rare diseases due to numerous mutations in the genes coding for CLC1 (CLCN1 gene) and for the α subunit of NaV1.4 (SCN4A gene).

Congenital myotonias related to the CLCN1 gene (chloride channelopathies) include Thomsen myotonia congenita, Becker myotonia congenita and myotonia levior.

Congenital myotonias related to the SCN4A mutations (skeletal muscle sodium channelopathies) include myotonia permanens, myotonia fluctuans acetazolamide-responsive myotonia, paramyotonia congenita and hyperkalemic periodic paralysis with myotonia (1 -3).

The phenotypic spectrum of CLC1-related CMs is very variable, ranging from milder forms, characterized only by myotonia (more often AD forms), to more severe forms, in which myotonia can be associated with myopathy (more often AR forms). The forms of CM from SCN4A mutations tend to be less severe (even if in permanens form, myotonia can be severe and cause respiratory distress) but often, due to the onset of pain and/or fatigue, they can be disabling. Given the large number of genetic mutations and the low incidence, knowledge about the natural history and phenotypic variability (including the association of myopathy) of these congenital myotonias is limited (1-3).

Myotonic dystrophies (DM), indicated as type 1 (or Steinert's disease) and type 2 (or proximal myotonic myopathy - PROMM), are both transmitted with AD modality. They represent the most frequent form of muscular dystrophy in adults and are caused by the unstable expansion of nucleotide repeats (CTG triplets and CCTG quadriplets) in the genes coding for myotonic dystrophy protein kinase (DMPK) and for the zinc finger protein 9 or cellular nucleic acid-binding protein (ZNF9/CNBP), respectively in DM1 and DM2 (4-5).

Myotonia is not the only symptom or necessarily the dominant symptom in the different forms of MS and, by itself, does not allow the differential diagnosis among the different syndromes. Moreover, while in myotonia congenita the myotonic phenomenon may be directly related to the genetic mutations (mutations

in voltage-gated ion channels) (1-3), in myotonic dystrophies (DM 1 and DM2) different mechanisms must be taken into account (4-6).

Since the principal objective of this project is the evaluation of genotype-phenotype correlation in DM1 patients, the characteristics of this syndrome are described in more detail, in consideration of the differential diagnosis with other MS and particularly with DM2.

MYOTONIC DYSTROPHIES

From a clinical point of view, both DM1 and DM2 are characterized by progressive muscle degeneration and multisystem involvement of various organs and systems. The core pattern of the clinical presentation includes muscular weakness (both skeletal and smooth muscle involvement) with myotonia and dystrophy, cardiac involvement (with conduction defects and cardiomyopathy), respiratory disturbances, ocular disturbances, endocrine and gastrointestinal disorders. Moreover, a central nervous system dysfunction (CNS) with sleep disorder, cognitive impairment, behavioural and psychiatric disorders is now widely recognized; a peripheral nerve involvement has also been described (4-7).

The wide clinical spectrum of DM seems to be determined, at least in part, by their genetic background: in both disorders the unstable expansion of nucleotide repeats in untranslated DNA regions leads to accumulation of mutant RNA aggregates, with consequent mis-splicing of downstream effector genes that affects almost all cells and organs of the human body (4). However, it is hypothesized that other mechanisms, not yet fully elucidated, are involved in DM pathogenesis (5, 6).

The dynamic nature of these genetic mutations, together with somatic mosaicism, leads to an extremely heterogeneous phenotype with multisystem involvement and unclear genotype-phenotype correlations.

Large-scale prospective studies taking into account the multi-organ involvement of DM and the evaluation of genotype-phenotype correlations using standardized methodologies are still lacking.

I. MAIN NEUROLOGICAL FEATURES OF MYOTONIC DYSTROPHIES

Myotonic Dystrophy type I (DM1 or Steinert's disease)

This is the most frequent form of myotonic dystrophy, with an incidence of approximately 1/8000 people (1).

There is not a standard classification system for DM1 patients, and studies used different classifications: sometimes according to severity (correlating this to the number of CTG repeats: mild, classical, congenital), other times according to age of onset (congenital, infantile/juvenile, adult type).

The French Myotonic Dystrophy Clinical Network created a five-grade classification system to provide a standard framework and to improve research and study of DM1 (8, 5).

According to these authors, patients are classified into the following age groups of disease onset:

- **Congenital DM1 (cDM1)** (onset from birth to 1 month): patients with congenital DM1 have usually large CTG expansions (>800-1000 CTG). Characteristically, these large expansions are caused by maternal transmission, but cDM1 with paternal transmission have been also described (9, 10-13). It is the most severe form, characterized by prenatal symptoms (reduced foetal movements and polyhydramnios), severe

generalized weakness after delivery with hypotonia and frequent respiratory distress. Surviving infants experience gradual improvement in motor function, almost all cDM1 children are able to walk (14-15). Myotonia is absent at birth, appears in a small percentage of patients by 5 years of age, and increases by 10 years of age. Cognitive and motor milestones are delayed. Speech disturbances, poor motivation, and inability to concentrate are symptoms of attention deficit hyperactivity disorder (ADHD) and autism spectrum disorders, which may be observed as the child grows (16, 17, 18).

- **Childhood DM1** and **Juvenile DM1**(2, 3, 18): commonly, there is an expansion of CTG-repeats of more than 800 CTG repeats. Disease onset is at 1–10 years of age for childhood and 10–20 years of age for the juvenile form. These two clinical groups are characterized by predominant cognitive and behavioural features that are not accompanied by conspicuous muscle impairment (19). The principal complaints in early childhood are speech and learning difficulties and psychiatric symptoms, such as anxiety disorder, mood disorder, and attention-deficit-hyperactivity disorder (17). The diagnosis of childhood DM1 is often missed due to the lack of clearly neurological symptoms. In adolescence, patients may show typical muscular and non-muscular symptoms of adult-onset DM1, like distal weakness, clinical myotonia, cardiological or gastrointestinal symptoms (9).
- **Adult DM1** (onset from 21 to 40 years): disease onset is difficult to determine because patients are often unaware of symptoms. The most common initial symptom is myotonia, mainly affecting the fingers (grip myotonia), tongue and jaw causing problems with talking, chewing and swallowing; when muscle weakness worsens, myotonia becomes less evident. Myopathy is characterized by weakness and muscle wasting with preferential involvement of cranial, trunk, and distal limb muscle, mainly involving finger flexors, wrist flexors, and foot extensors with foot drop. All cranial muscles are potentially affected, with ptosis, wasting of temporalis and masseter, tongue and facial weakness; the speech can be nasal and slurred, sometimes causing chewing and swallowing difficulties. The neck flexors are affected early. Diaphragmatic weakness may occur before there is any weakness of the limb girdle muscles (7).

  Early onset posterior subcapsular cataracts develop in most patients. Cardiac involvement is common including conduction abnormalities and arrhythmias and, in some patients, dilated cardiomyopathy. Pulmonary dysfunction can present with a restrictive syndrome and with hypoxemia/hypercapnia.

  Central nervous system (CNS) involvement can include cognitive, psychiatric and sleep disorders, mainly sleep breathing disorders and daytime sleepiness. Gastrointestinal tract involvement (with irritable bowel syndrome, symptomatic gall stones and gamma-glutamyl-transferase elevations) and endocrine abnormalities (including testicular atrophy and insulin resistance) are also frequently reported (4).
- **Late onset/asymptomatic** (onset after the age of 40 years)**:** small CTG expansions (in the range of 70 to 100 repeats) are usually associated with mild weakness, myotonia, and cataracts that begin after age 40 (4).

Myotonic dystrophy type 2 (or proximal myotonic myopathy – PROMM)

DM2 appears to have a lower prevalence than DM1, but large-scale population studies are lacking. It is generally accepted that no correlation exists between repeat size and the clinical picture. There are no distinct clinical subgroups in DM2, in particular there is no evidence of a congenital or childhood form. Clinical presentation comprises a continuum ranging from early adult-onset severe forms to very late-onset mild forms that are difficult to differentiate from normal aging.

Symptoms of DM2 usually begin between 20 and 50 years of age, often with early onset cataracts, grip myotonia, muscle stiffness and pain, especially in the arms and upper lower back; pain in these patients seems to be muscular in origin but not necessarily connected to myotonia, and a prior diagnosis of fibromyalgia is relatively common.

Muscular weakness is typically localized in hip flexors, hip extensors, long flexors of the fingers and neck flexors; distal limb muscles are usually spared until later in the course and facial weakness is usually mild. Muscle wasting is less pronounced and some patients exhibit hypertrophy of calf and thigh muscles.

Myotonia seems to be usually mild to moderate or even absent in many DM2 patients, impacting only minimally their quality of life; as a consequence, the majority of DM2 patients remain undiagnosed even in clinical centres with considerable experience (20). Few patients may, however, display severe myotonia and in some of these cases additional mutations in ion channel genes CLCN1 and SCN4A have been identified (41, 42). Multiorgan involvement, similar to that described in DM1 but usually less severe and frequent, has been described, with cardiac conduction disturbances, endocrine abnormalities (hypogonadism, insulin resistance, hypothyroidism, hyperlipidemia), gastrointestinal manifestations (trouble in swallowing and constipation), respiratory abnormalities and sleep disorders (in particular obstructive sleep apnoea) (20-22). The cognitive and behavioural picture is similar to the adult form of DM1, although less severe; patients with DM2 are characterized by apathy, "avoidant" personality and a significant impairment in frontal lobe function (especially in executive functions) (20), but their IQ and test scores remain within normal limits.

Conventional brain MRI findings can be entirely normal; however, in advanced stages or more severe cases, diffuse white-matter changes can be present, although less pronounced than in DM1 (23).

II. GENETIC ASPECTS OF MYOTONIC DYSTROPHIES

Myotonic Dystrophy type I (DM1 or Steinert's disease)

DM1 is an autosomal dominant disorder caused by unstable expansion of triplet repeat (CTG) in the 3' untranslated region of the Dystrophia Myotonica Protein Kinase (DMPK) gene.

According to the number of (CTG)n repeats, patients affected are divided into 3 main genetic classes:

- **E1**: 50-150 repeats CTG, usually with a **mild clinical phenotype**;
- **E2**: 150-1000 repeats CTG, usually with a **classic phenotype** with a wide span from mild to severe symptoms;
- **E3**: > 1000 repeats CTG, mainly associated with the **congenital form.**

Repeat lengths of 38–49 CTG are considered **premutation alleles**, whereas 51–100 repeats are **proto-mutations**, both of which show increased instability toward expansion: these patients are asymptomatic or present mild symptoms, such as cataracts, but are at risk of having children with larger, pathologically expanded repeats (24). In fact, repeat mutations are dynamic gene defects that show instability: different numbers of repeats in different tissues (somatic mosaicism) increase over time in the same individual and across generations, the so called "anticipation phenomenon" in which disease severity increases and age of onset decreases from one generation to the next. Children with congenital DM1 almost always inherit the expanded mutant DMPK allele from their mother; however, anticipation may be also seen in patients who inherit a smaller expanded CTG repeat from their father (25, 26). A decrease in the CTG repeat size during transmission from parents to child can also occur, in about 6.4% of transmissions, most frequently during paternal transmission (10%) (27, 28).

The length of CTG expansion depends on both meiotic and somatic instability. Previous studies demonstrated that CTG expansion is larger in brain, muscle and skin cells compared to peripheral blood leukocytes (29). The DM1 expansion seems to be more unstable in non-dividing cells of skeletal muscle, heart, and brain than in proliferating cells of the hematopoietic system.

The bias towards expansion is the cumulative effect of many expansion and contraction events that seem to be coupled with DNA repair or transcription rather than be dependent on DNA replication. The tendency of the repeat tract to expand or contract seems to be a function of its primary sequence, which enables formation of secondary hairpin structures, and its genomic location, including flanking sequences and the distance from the origin of replication. Around 5% of DM1 families have sequence interruptions within the CTG repeat. Most commonly these are (CCG)n or (GGC)n triplets interspersed among CTG triplets. It appears that sequence interruptions tend to stabilize the repeat tract reducing the rate of expansion in affected tissues and, in some cases, may lead to variant phenotypes with generally less severe symptoms (30-33, 4).

The dynamic characteristics of this mutation and the somatic mosaicism make genotype-phenotype correlation difficult in these patients. Moreover, the correlation between CTG repeat size and the severity of the disease can be observed in the blood but not in other organs (for example, in muscle repeat sizes are larger but there is no correlation with muscle weakness). CTG expansions that are slightly larger, comprising 70-90 repeats, are usually associated with mild symptoms that begin after age 40; at the other end of the spectrum, congenital DM1 is usually associated with expansions of more than 1,000 repeats. However, between these two extremes, predictions about the progression of the disease based on CTG-repeat size should be made very carefully (7).

Genetics of DM2 (or proximal myotonic myopathy – PROMM)

Myotonic dystrophy type 2 is an autosomal dominant disorder caused by unstable expansion of repeat (CCTG)n in the first intron of the cellular nucleic acid binding protein/zinc finger 9 (CNBP/ZNF9).

In DM2 the (CCTG)n repeat is a part of the complex repetitive motif (TG) n(TCTG)n(CCTG)n. In contrast to the DM1, the DM2 associated (CCTG)n repeat tract is generally interrupted in healthy range alleles by one or more GCTG, TCTG or ACTG motifs, while it is typically uninterrupted in the expanded alleles (34, 35).

In DM2 the expansion ranges from 55 to 11,000 CCTG-repeats; in contrast to DM1 the mutation usually contracts in the next generation, being shorter in children (34, 36, 37, 20). This may explain some distinct features of DM2 such as the missing of a congenital form, the lack of anticipation and the later disease onset (34). Similar to DM1, these expansions are extremely unstable, giving rise to intra-tissue, inter-tissue, and cell-type variability with somatic mosaicism over a patient's lifetime (37-39); the size of the CCTG repeat appears to increase over time in the same individual, making the genotype-phenotype correlation difficult to establish (20).

Recent studies described in DM2 patients a co-segregation of heterozygous recessive CLCN1 and SCN4A gene mutations. These patients showed more severe muscle stiffness and more severe myotonia than those having exclusively the CNBP expansion, leading to easier diagnosis (34, 40-42).

III. PATHOMOLECULAR MECHANISMS

Both DM1 and DM2 are caused by dynamic and unstable expanded microsatellite sequences in the non-coding regions of the genes DMPK and CNBP, respectively.

It is now clear that the gain-of-function RNA mechanism is the most accredited etiopathogenetic hypothesis explaining at the same time the multisystem involvement and the phenotypic heterogeneity of DM.

The expanded CUG and CCUG RNA form hairpins, imperfect double-stranded structures that lead to dysregulation of two important RNA-binding proteins: muscleblind-like 1 (MBNL1) and CUG-binding protein 1 (CUGBP1) (43-48). These proteins are antagonist regulators of alternative splicing of various genes, and alterations of their functional levels in DM tissues result in the reversion to foetal splicing patterns for several mRNAs, explaining the multisystem phenotype (6, 7).

The **MBNL proteins** appear to play a prominent role in DM pathogenesis since each of the three MBNL isoforms (MBNL1, MBNL2 and MBNL3) are sequestered by CUG RNAs in the cell nuclei. MBNL1 plays the predominant role in alternative splicing regulation in skeletal and cardiac muscle, while MBNL2 serves a related function in the central nervous system (49-51).

CUGBP1 contributes to the induction of the embryonic splicing patterns; it is a member of the family of CELF (CUGBP, Elav-like family) proteins, a multifunctional protein overexpressed in myoblasts, skeletal muscle and heart tissues (53); it functions in the nuclei and cytoplasm and regulates splicing, stability and translation of RNAs (52, 54-56). CUGBP1 cellular localization depends on its phosphorylation status. CELF1 steady-state concentrations are upregulated by hyperphosphorylation via different signalling kinases, including protein kinase C (PKC), v-akt murine thymoma viral oncogene homolog 1 (AKT1), cyclin D3 (CCND3), cyclin-dependent kinase 4 (CDK4), glycogen synthase kinase 3 beta (GSK3B), and double-stranded RNA-dependent protein kinase (PKR). The activation of the Akt pathway increases CUGBP1 phosphorylation altering the transition from proliferating myoblasts to differentiated myotubes (57, 4). On the other hand, DM cells show decreased activity of cyclin D3-cdk4, another kinase that phosphorylates CUGBP1, resulting in higher levels of unphosphorylated CUGBP1, which forms inactive complexes with

eIF2a (CUGBP1-eIF2a) affecting translation of mRNAs required for myoblast differentiation. These inactive complexes containing CUGBP1 accumulate in the cytoplasm of DM cells in stress granules.

Sequestration of MBNL proteins in mutant RNA nuclear foci and increased CELF steady-state protein levels, result in abnormal expression of embryonic isoforms in adult tissues.

A recent study summarized all the transcripts involved in the mis-splicing mechanisms in DM1 brain, skeletal and cardiac tissues (58-75). Among the transcripts altered in DM1 brain tissue the authors reported: the microtubule-associated protein tau (MAPT gene, strongly associated with progressive appearance of neurofibrillary tangles composed of intraneuronal aggregates of hyperphosphorylated tau protein in DM1 patients brain samples), the amyloid β precursor protein (APP gene, whose defects have been associated with the development of some tauopathies, in particular Alzheimer Disease), the NMDA receptor 1 (NMDAR1 gene, which plays a role in neuronal plasticity and excitotoxic damage when over-activated), MBNL2 and MBNL1 proteins (implicated in splicing defects). Among the transcripts altered in DM1 skeletal muscle, the same authors described the role of MBNL1 (the main alternative splicing regulator in skeletal muscle), the pyruvate kinase M2 (PKM2 gene, responsible for metabolism in skeletal muscle), the muscle chloride channel (CLCN1 gene, responsible for ion conductance and excitability), the dystrobrevin-α (DTNA gene, responsible for the sarcolemma stability), the calcium channel CaV1.1 (CACNA1S gene, which plays a central role in excitation–contraction coupling), the bridging integrator 1 (BIN1 gene, this protein is required for the biogenesis of muscle T tubules, which are essential for excitation–contraction coupling), the ryanodine receptor 1 (RYR1 gene, implicated in muscle contraction/relaxation cycle), SERCA1 (ATP2A1 gene, whose dysfunction leads to impaired intracellular calcium homeostasis and muscle degeneration), fast troponin T3 (TNNT3 gene, involved in the maintenance and mechanical support of the sarcomere during contraction), dystrophin (DMD gene, fundamental for membrane integrity), calpain 3 intracellular protease (CAPN3 gene, responsible for the cleavage of a big range of proteins implicated in the sarcolemma structure), myomesin 1 (MYOM1 gene, responsible for sarcomeric M-band integrity), skeletal muscle insulin receptor (a lower-response insulin receptor, which causes a decreased metabolic response to insulin that leads to an unusual form of insulin resistance in DM1 patients). Finally, among the transcripts altered in DM1 cardiac muscle the following were described: the cardiac voltage channel NaV1.5 (SCN5 gene, whose mis-splicing affects conductance of the channel, leading to slowing of the normal conduction), SERCA2 (ATP2A2 gene, which contributes to the calcium influx dysregulation in cardiac muscle, leading to complications in cardiac conduction), troponin 2 (TNNT2 gene, the foetal isoform of this protein confers different calcium sensitivity to the myofilament, affecting the contractile properties of muscle contributing to the reduced myocardial function and conduction abnormalities) and TTN and ZASP/LDB (responsible for morphological abnormalities of the cardiac fibres).

However, there is no direct evidence of a cause-effect relationship between symptoms and mis-splicing, and it is now clear that spliceopathy may not fully explain the multisystem disease spectrum; thus other molecular mechanisms were proposed. A novel molecular mechanism that may contribute to the pathogenesis of myotonic dystrophies has been described by Zu and collaborators (77). RNA transcripts containing expanded

CAG or CUG repeats can be translated in the absence of a starting ATG, and this noncanonical translation, called repeat associated non-ATG translation (RAN-translation), occurs across expanded repeats in all reading frames to produce potentially toxic homo-polymeric proteins. RAN-translation products appear to be toxic to cells and may contribute to DM1 and DM2 pathology and may also be responsible for some of the CNS features of DM (58, 77). Moreover, some authors observed that miRNAs (small, noncoding RNA modulating gene expression at the posttranscriptional level) expression and intracellular distribution are deregulated in many human diseases (58, 78-82). Both in DM1 and DM2 it has been demonstrated that the highly regulated pathways of miRNA are altered in skeletal muscle, potentially contributing to myotonic dystrophy pathogenetic mechanisms (80-82). A group of microRNAs known as myomiRs (miR-1, miR-133a/b and miR-206) as well as other microRNAs, have been extensively studied in peripheral blood plasma of DM1 patients, correlating with skeletal muscle strength, and they have been proposed as non-invasive biomarkers of the disease. However further studies are needed (83, 84, 85).

IV. EXTRA-MUSCULAR CLINICAL ASPECTS OF MYOTONIC DYSTROPHIES

1. CARDIOVASCULAR INVOLVEMENT

Both DM1 and DM2 are associated with an increased risk for conduction abnormalities (atrio-ventricular block, left anterior fascicular block, right and complete left bundle branch block leading to permanent pacemakers implantation), atrial arrhythmias (atrial flutter and atrial fibrillation), ventricular arrhythmias, cardiomyopathy (dilated cardiomyopathies), heart failure (leading to permanent defibrillation device implantation) and, less frequently, coronary heart disease; electrocardiographic alterations (like PR interval length ≥240 ms or QRS ≥120 ms) have been proven to be at higher risk incidence of cardiac events (86).

In DM2 patients, cardiac involvement is similar to those observed in DM1 but occurs less frequently, as demonstrated by a recent observational case–control study on a large cohort of DM2/DM1 patients (22, 89, 90); cardiomyopathy might occur in about 3% of DM2 patients (91, 92, 93).

Cardiac arrhythmias can precede the onset of neuromuscular symptoms and constitute the second leading cause of death after respiratory failure in DM1 patients (87); not every patient will develop cardiac abnormalities, however as they potentially affect >50% of subjects, regular screening by a cardiologist familiar with neuromuscular diseases is recommended (88).

Some studies described a correlation between CTG expansion, the extent and rate of progression of cardiac disease, especially with conduction disturbances (94-96, 97-98, 99-107); however, this was not universally observed (108-112). Cardiac abnormalities appear to be more consistently associated with age, disease duration and male gender than with CTG repeats (113, 97, 22). Moreover, peripheral blood leucocyte DNA can underestimate CTG repeat lengths relative to skeletal and cardiac muscle DNA, where expansion lengths may be up to 13-fold longer (114, 115, 97).

The precise mechanisms by which DM1 promotes cardiac conduction system dysfunction are not well understood. Cardiac fibrosis and fatty infiltration most commonly affect the His–Purkinje system but may also involve the sino-atrial and atrioventricular (AV) nodes, providing a substrate for conduction block,

ectopic activity and re-entrant arrhythmias (95, 97). Abnormal splicing of the SCN5A gene has also been implicated in cardiac conduction system disease (116). In addition, upregulation of NKX2.5 has been suggested to play a role in cardiac dysfunction (117). Similar mechanisms have been suggested for DM2 (88). Autonomic dysfunction may further complicate blood pressure response and embolic risk (118); a mixed, predominantly parasympathetic cardiac dysfunction occurs, without apparent correlations with peripheral neuropathy or length of CTG repeat expansion (119). Moreover, respiratory involvement, with chronic hypoxemia and apnoea, provides a substrate for episodes of irregular heart rhythms that may be worsened with inadequate ventilatory nocturnal support (120); some authors suggested that restrictive syndrome was an independent prognostic factor of cardiac events in a multivariate Cox analysis (107).

On the other hand, cardiac comorbidities may accelerate respiratory impairment, through impaired respiratory muscles perfusion (121,122).

To conclude, patients with DM should be instructed to report events including palpitations, syncope, near syncope, dizziness, and light-headedness; however, since arrhythmias may be asymptomatic, regular monitoring including ECG, Holter ECG and echocardiogram are recommended (88). Cardiac magnetic resonance imaging (CMR) can facilitate the detection of systolic dysfunction, left or right ventricular hypertrophy, ventricular dilatation, areas of fatty infiltration, fibrosis and occasionally non-compaction (123).

2. RESPIRATORY ASPECTS

In DM1, mortality is mainly due to respiratory problems, usually resulting from respiratory failure or aspiration. The predominant respiratory function abnormality in DM patients is a restrictive ventilatory pattern, resulting in a reduction of maximal inspiratory pressure (MIP), maximal expiratory pressure (MEP), forced vital capacity (FVC) and forced expiratory volume in the first second (FEV1) (105). Arterial blood gas abnormalities with progressive hypoxemia (defined as arterial oxygen partial pressure PaO2 <80 mmHg) and hypercapnia (defined as arterial carbon dioxide partial pressure PaCO2>45 mmHg) are also frequently reported. A fall of FVC in the supine position of more than 25% comparing the sitting position indicates a significant diaphragmatic weakness and probably nocturnal hypoxemia; with further progression of diaphragmatic involvement and the continuing decline in FVC, patients become more susceptible to sleep-breathing disorders, which appear first during REM sleep (134).

Abnormal respiratory function tests (FVC, FEV1, MIP and MEP values) were found to be associated with severity of muscle disease (135-139, 107, 122), age, BMI (140, 136, 138), duration of muscle symptoms (138), hypercapnia (141, 142, 148), cardiac conduction abnormalities (107, 136, 122) and CTG repeat length (127, 136, 138-140, 122); other studies, on the contrary, found no relationship between CTG repeat length and respiratory dysfunction (141, 143-145). Earlier reports showed that in DM1 patients chronic hypercapnia increased in relation with the severity of muscular disability (107, 136, 146), the CTG repeat length (127) and spirometry parameters (146, 148), while most studies did not confirm the latter association (127, 130, 141, 142, 146, 148). Poussel et al. (133) observed that postural change in FEV1 was the only significant predictor of daytime hypercapnia. Other authors observed a reduced ventilatory response to CO2 independent

of respiratory muscle weakness (130). The finding of a relative preservation of CVF in hypercapnic patients, suggested that abnormalities of central ventilatory control participate in chronic alveolar hypoventilation in DM1 patients (127, 146). In fact, breathing disorders in DM1 have a complex aetiology, combining both peripheral respiratory involvement (respiratory muscle weakness and myotonia) and central respiratory drive dysfunction (105, 127, 129). This hypothesis was supported by the finding of severe neuronal loss in various medullary nuclei linked to respiratory function in DM patients with alveolar hypoventilation (132).

Few data are available regarding DM2 patients and respiratory disorders; respiratory involvement is reported in about 6–15% of cases (129, 131, 149).

Only some studies investigated changes in respiratory function with time. The largest study was published by Thil et al. (150), reporting annual declines in all respiratory function parameters over a follow-up period of 5 years. While the majority of studies indicate a progressive decline in respiratory function over time, the trajectory of decline (e.g. linear or non linear) remains unclear (105). In a follow-up study of 2 years conducted on 33 DM1 patients, Mazzoli et al demonstrated, consistently with previous works, that large CTG expansion significantly affected the risk of progression in muscular involvement and that FVC was a reliable indicator of respiratory decline; moreover, worsening in MIRS score and CTG expansion size were independent predictors of NIV start (122). However, a recent study found that greater CTG repeat size, higher MIRS rating, and longer disease duration were all correlated with lower baseline CVF, but not with annual rate of change, suggesting that decline is not due to progressive muscle impairment alone; authors explored also the impact of NIV compliance on the rate of decline of CVF and found that NIV compliant patients experience slower rates of FVC decline compared to non-compliant patients, supporting the utility of NIV (138).

It happens frequently that myotonic patients do not relate their symptoms to respiratory failure (129), thus a respiratory symptoms checklist ("RESPICHECK") was recently proposed (151). Consensus-based care recommendations for pulmonologists treating adults with DM1 have recently been drawn up (152), recommending an accurate screening of respiratory disease symptoms that may indicate nocturnal hypoventilation (poor sleep, morning headaches, orthopnoea, excessive daytime sleepiness), obstructive sleep apnoea (snoring, witnessed apnoeas, gasping/choking from sleep, excessive daytime sleepiness), decreased respiratory reserve (dyspnoea, orthopnoea, tachypnoea, fatigue, exercise intolerance), ineffective cough (decreased ability to cough, coughing when eating or drinking, choking, history of excessive frequency and duration of chest infections). In addition, it was recommended that, even at the initial assessment, a complete pulmonary screening with spirometry (including FVC and FEV1 in a sitting and supine position, MIP, MEP), daytime blood gas analysis or end-tidal and/or transcutaneous CO2 analysis, nocturnal oximetry/capnography or polysomnography (transcutaneous capnometry most accurately reflecting nocturnal CO2 changes) should be included. As recommended by the 207th ENMC workshop, NIV therapy should be prescribed when symptomatic chronic respiratory insufficiency is combined with daytime hypercapnia ($PaCO_2$>50mmHg), FVC <50% plus MIP <60cmH2O over 3 measurements, or evidence of nocturnal

hypoventilation (including a rise in PaCO2 of >8mmHg between evening and morning arterial blood gas analysis, or overnight oximetry demonstrating significant hypoxia) (129, 152).

3. AUTONOMIC NERVOUS SYSTEM (ANS) INVOLVEMENT

Respiratory impairment, heart rhythm conduction disturbances, orthostatic hypotension, disorders of sweating, abnormal gut movements, genitourinary dysfunction and pupillary abnormalities have been reported in DM patients. These symptoms can be explained either by a muscular impairment (smooth or cardiac muscle) or by an ANS dysfunction (central or peripheral).

Some anatomopathological studies seem to support a possible involvement of ANS in DM. Ono et al observed a loss of catecholaminergic neurons in the medullary reticular formation in DM patients with alveolar hypoventilation, supporting a possible central nervous system involvement in respiratory dysfunction. More recently, Kuru et al described the presence of neurofibrillary tangles (NFTs) in sympathetic ganglions of 5/7 MD patients compared to controls but were unable to obtain detailed information on the autonomic function of these patients (132, 186). Clinical and neurophysiological studies, using cardiovascular reflex tests and power spectral analysis (PSA) of heart rate variability (HRV) (a technique that may better reflect autonomic cardiovascular influences) investigated cardiovascular autonomic function on small groups of DM patients, with conflicting results; the different techniques used in these studies do not allow a comparison between the results obtained (153). To the best of our knowledge no study examined the autonomic small nerve fibre function in DM1 patients.

- **Cardiovascular ANS** (cANS) function and its relationship with heart involvement in DM patients remain unclear (154, 155). Cardiovascular reflex tests were first investigated demonstrating only minor signs of parasympathetic dysfunction (156-158). Later studies, using autoregressive power spectral analysis (PSA) of heart rate variability (HRV) demonstrated an age-related decline in heart variability indices, suggesting a reduced parasympathetic tone together with a slight increase in sympathetic activity (159-161). Di Leo et al studied 23 DM1 patients with cardiovascular autonomic tests, nerve conduction study, heart and breathing evaluation; 14/23 patients showed some alterations (the most frequent being a significant reduction of heart rate variability during deep breathing) and 1 patient had a definite autonomic damage, without correlation with heart abnormality, electro-neurography or spirometry parameters. Moreover, using PSA, they observed a reduction of supine low-frequency band, a marker (although not exclusively) of sympathetic activity, that was inversely correlated to disease duration (154). Rakocević-Stojanović et al, using the same neurophysiological techniques, observed the presence of a sympathetic dysfunction and vagal predominance in DM1 patients, without significant correlations with the severity of disease or CTG repeat length (162). Similar minimal abnormalities of cardiovascular autonomic function were found in patients with DM2, especially those with cardiac arrhythmias (163). Magrì et al found significantly lower HRV and higher QT variability index (QTVI) (a marker of temporal dispersion in cardiac repolarization closely associated with sudden cardiac death risk) in DM1 patients compared to controls; moreover, they observed a significant relationship between this index and both PR interval length and CTG repeat expansion size, which are well-

established predictors of disease severity and arrhythmic risk (164). A subsequent study observed in both DM1 and DM2 patients compared to controls, an increased QT interval dispersion (QTd) on standard electrocardiography, and a reduction of heart rate turbulence (HRT) parameters on 24h Holter monitoring; no relationship was found between disease duration or neurological status and HRV, HRT and QTd parameters. The authors concluded that diminished HRT in both types of DM may suggest minor parasympathetic dysfunction or other unknown dysfunction associated with baroreflex sensitivity, not confirming a certain role of cANS dysfunction in the genesis of cardiac abnormalities (155). More recently Rossi et al documented the presence of RNA foci in the nerve biopsy of 2 related DM2 patients with an atypical disease onset characterized by dysautonomic symptoms; however, the cardiac scintigraphy with I-mIBG (metaiodobenzylguanidine), which allows detecting abnormalities in the cardiac sympathetic innervation, was normal, suggesting that the aetiology of arrhythmias could be ascribed to focal cardiac fibrosis (165).

Tobaldini et al studied cardiac autonomic control (CAC) during sleep in patients with DM1 compared to controls; they found in DM1 patients preserved cardiac autonomic modulation, with a vagal predominance during non-rapid eye movement (NREM) sleep and a sympathetic predominance during rapid eye movement (REM) sleep; obstructive sleep apnoea syndrome (OSAS) did not affect the CAC, but was associated with a reduction in HRV (which is a marker of the ability of autonomic control to respond to stressors stimuli) during the whole sleep-wake cycle, and sympathetic predominance and low vagal modulation during wakefulness (166).

- **Gastrointestinal function**

DM has been reported in the literature as a cause of severe colonic impaction and intestinal pseudo-obstruction, although this complication appears to be rare. Intestine dysmotility may result either from a dysfunction of the intestinal smooth muscle (168) or from a dysfunction of enteric nervous system; the fundamental nature of the disease process in DM is still debated (167).

- **Pupillary function**

Few studies examined pupillary abnormalities in DM patients. A sluggish pupillary response to light and near vision was reported, as well as tonic responses termed 'myotonic pupils'. Earlier studies showed no defect in the sympathetic pathways, suggesting that miosis and sluggish pupillary responses could be explained by a possible midbrain lesion (169). Spaide found defective pupillary dilation in darkness in two patients and proposed a preganglionic sympathetic lesion (170). Alon et al found delayed pupillary cycle time in nine out of 18 DM1 patients; as the results of blink reflex seemed to exclude a brainstem lesion, the authors examined a possible autonomic nervous system involvement testing cholinergic and adrenergic sensitivity of the pupils (using respectively a pilocarpine solution 0.1% and two drops of phenylephrine 1%). None of patients, however, exhibited evidence of cholinergic supersensitivity, confirming previous observations. The authors concluded that structural damage to the iris smooth musculature was the most likely explanation of the prolonged pupillary cycle time (171). Den Heijer et al found that latency of the pupillary light reflex was not different comparing DM1 patients from controls, but the time to reach peak velocity of contraction was

significantly longer in the DM1 group; these authors argued that these results suggested a dysfunction of iris smooth muscle rather than autonomic nervous system dysfunction (249).

4. PAIN

In all the myotonic syndromes one of the most frequently reported symptom is pain, which can have a deleterious impact on quality of (QoL). In DM1 patients musculoskeletal pain or myalgia may be present in some patients, but in general is often overlooked by patients and physicians, who often have a greater focus on other symptoms (172, 173). Pain is more frequent in DM2 patients (50-80% of cases), sometimes as a main symptom; in these patients it appears to be most often located symmetrically in the proximal limbs, it is sometimes reported to be exercise-related, temperature-modulated or palpation-induced, and has no consistent relationship with the severity of myotonia (174-177). Peric et al observed that average pain intensity was strongly associated with longer disease duration and worse cognition in DM1 patients, while in DM2 patients it was associated with female gender and emotions (172).

The pathophysiology of pain in DM is poorly understood. Peripheral and central sensitization are likely to play a prominent role in chronic musculoskeletal pain. A recent study using quantitative sensory testing (QST) described mechanical hyperalgesia in patients with DM2 compared to healthy controls, which is indicative of the presence of peripheral sensitization (172, 175, 178). Boland-Freitas et al using thermal threshold QSR evaluated small sensory fibre function (Aδ and C-fibre) of 16 DM1 patients without large fibre neuropathy; they observed higher warm detection thresholds in hand and foot in DM1 patients compared to controls, while cool detection thresholds were lower, suggesting that a subclinical small sensory fibre dysfunction occurs in DM1 patients (179).

5. PERIPHERAL NERVOUS SYSTEM IMPAIRMENT

Nerve conduction studies (NCS) reported a predominantly subclinical involvement of the peripheral nerve in 14-54% of DM1 patients, the most frequently described was a demyelinating and motor polyneuropathy.

Clinical manifestations are not typical at early stages of the disease, but may occur in later stages, contributing to balancing impairment and increased risk of falls (180, 181). Recent neurophysiological studies suggest similar findings in DM2 patients (165, 183, 184).

Whether peripheral neuropathy is caused by metabolic and endocrine dysfunctions or is a multisystem manifestation of DM is a matter of debate. Some authors found no correlations with age, duration of symptoms or CTG-repeat size (180, 182), suggesting that the affection of peripheral nerve system is secondary to metabolic and endocrine dysfunctions. On the other hand, Peric et al described a possible correlation with male gender, age, disease duration and certain metabolic parameters (higher BMI, dyslipidemia) suggesting that in DM neuropathy may be a consequence of altered alternative splicing of certain axon or myelin proteins (181, 182). In line with this observation recently Rossi et al documented the presence of RNA foci in the nerve biopsy of 2 related DM2 patients with an atypical disease onset characterized by dysautonomic symptoms (165).

To the best of our knowledge to date only one study evaluated small nerve fibre dysfunction in a small number of DM1 patients using Laser evoked potentials (LEP) and skin biopsy (185).

6. CENTRAL NERVOUS SYSTEM (CNS) INVOLVEMENT

SLEEP DISORDERS: Myotonic dystrophy type 1 (DM1) represents the chronic neuromuscular disease with the most prominent sleep disorders, including excessive daytime sleepiness (EDS), sleep apnoea, periodic leg movements during sleep, and rapid eye movement sleep dysregulation.

Excessive daytime sleepiness (EDS)

Increased daytime sleepiness and fatigue are among the most frequent non-muscular symptoms in patients with DM1 and DM2 (187-194). EDS can occur even before onset of muscle symptoms and may have potential adverse consequences, including reduced attention, vigilance and memory dysfunction, as well as deleterious impact on work, domestic responsibilities, social life, and quality of life (189, 195, 196). Moreover, patients affected by DM frequently experienced fatigue, equivocally defined as a lack of energy and feeling of exhaustion, which may be referred to as EDS (189, 194, 197-200).

DM1 patients rarely report EDS spontaneously, being mostly mentioned by family members. Diagnostic assessment of EDS is performed by means of standardized tests which quantify objective daytime sleepiness (like the Multiple Sleep Latency Test -MSLT), requiring sophisticated equipment and sleep training. There is still debate about the use of clinical interview and self-reported questionnaires (like Epworth Sleepiness Scale and Daytime Sleepiness Scale) for a subjective evaluation of EDS, because these instruments are not specific for DM patients and do not take into account possible confounding factors like fatigue and apathy (203, 191). EDS in these patients is often described as a persistent sleepiness unaffected by naps, the latter being frequently long, unrefreshing and without any associated dream content; sleepiness mainly occurs when attention is not being held or in monotonous situations in contrast to narcolepsy (191). Although some studies reported lower mean sleep latencies on the standardized MSLT in DM1 patients vs controls (190-192, 201), most studies did not find a positive relationship between daytime sleepiness complaints and MSLT results (187-189, 193). An often-reported finding during MSLT was the occurrence of sleep-onset REM periods (SOREMPs) similar to those seen in narcolepsy (187-189). The hypothesis of a dysfunction of hypothalamic hypocretin system was suggested by Martınez-Rodrıguez et al. (193) but was not confirmed by more recent studies (187, 204).

Some studies suggested the presence of EDS also in DM2 patients, even if Epworth Sleepiness Scale (ESS) Scores were not elevated (194, 205, 206). In the study by Romigi et al (207) mean ESS scores were not elevated in DM2 subjects, but mean Daytime Sleepiness Scale (DSS) scores were significantly elevated.

The pathogenesis of EDS in DM patients still remains unclear, however available data support the hypothesis that it could be a consequence of a central dysfunction of sleep regulation (188-196). Many factors can contribute to EDS in DM: an associated mood disorder like depression (as a result of brain dysfunction or a secondary reaction to the physical disability), sleep fragmentation, REM sleep dysregulation, sleep related breathing disorder (SRBD), chronic hypercapnia. However, previous studies did not find any association

between EDS and BMI, CTG repeat number, daytime pulmonary function, MSLT results or with polysomnography (PSG) findings including periodic limb movement (PLM), REM sleep characteristics, nocturnal respiratory events, mean O2 desaturation (188-190, 202, 203, 195, 196, 144), suggesting that DM patients have a primary hypersomnia. A study of 43 DM1 patients revealed that subjects with EDS (measured during Multiple Sleep Latency test) were characterized by more severe respiratory muscle weakness, lung volume restriction and higher levels of CO2; however, none of these parameters appeared to be good predictors of the occurrence of daytime sleepiness or sleep related breathing disorders (189).

Altogether, available data support the hypothesis that in DM a brain/brainstem disorder can lead to alteration of the sleep wake systems with central hypersomnia and REM sleep dysregulation (188).

EDS can be explained by a primary central nervous system (CNS) disturbance that may include metabolic-endocrine dysfunction (especially disturbance of the hypothalamic–pituitary–adrenal axis with low pulsatile secretion of cortisol and growth hormone) or abnormal proinflammatory cytokines secretion (interleukin-6 and tumour-necrosis factor-α). From an anatomopathological point of view, intracytoplasmic inclusions in the anterior and dorsomedial thalamic nuclei, neuronal loss and gliosis in the reticular formation, midbrain and pontine raphe, reduction of serotonin containing neurons in the dorsal raphe nucleus and in the superior central nucleus and a reduction of catecholaminergic neurons in the medullary reticular formation were described (132, 208, 332). Moreover, it was suggested that a direct toxic effect of the expanded CTG repeat may contribute to EDS in DM1, triggering alternative splicing of genes responsible for the sleep disturbances but that are still unknown (196, 187, 188, 209).

Limited data are available regarding imaging correlates of sleepiness and other sleep symptoms in DM. Sleepiness has been correlated with white-matter integrity in the superior longitudinal fasciculus and cingulum in DM1 patients and with brainstem and cerebellar peduncle changes in DM2 (210, 211, 212).

Sleep breathing disorders (SRBD)

A wide spectrum of SRBD can be observed in DM1 patients, including nocturnal hypoxemia, nocturnal hypoventilation, obstructive apnoeas and hypopnoeas as well as central apnoeas (188, 189, 202, 144, 148, 213-214). A polysomnographic study with a sample of 43 DM1 patients observed that a majority of subjects exhibited moderate to severe sleep apnoea and 30% of patients had severe sleep apnoea, with a predominance of obstructive events (189). A recent case control study confirmed that DM1 patients exhibited a higher apnoea index, central apnoea index, and AHI during NREM sleep than controls (188). Moreover, it has been observed that DM1 patients may exhibit clinically significant SRBD in the absence of daytime sleepiness and in the presence of normal daytime pulmonary functions. Few studies investigated the relationship between daytime respiratory function and sleep abnormalities using overnight sleep studies, with conflicting results; however, most studies concluded that SRBD cannot be predicted on the basis of clinical-neurological features and diurnal functional respiratory tests (105, 144, 148, 195, 202). For this reason, periodical evaluation by polysomnography should be mandatory to ascertain presence of obstructive sleep apnoea, periodic breathing or nocturnal hypoventilation (202).

SRBD have been described also in 38% to 64% DM2 patients (215, 216, 207, 217, 145). The pattern of SDB differs in DM2 as compared with DM1, since respiratory events are mostly obstructive, with a very few observations of central or mixed apnoea (145). SDB seems to be one of the leading causes of sleep disruption and EDS in DM2 (207).

SRBD may be explained by a dual mechanism: weakness and myotonia in facial, pharyngeal and laryngeal districts (that can justify the occurrence of obstructive respiratory events) and abnormalities of central control of ventilation. The hypothesis of an altered central control of ventilation is supported by findings of severe neuronal loss in various medullary nuclei linked to respiratory function in DM1 patients with alveolar hypoventilation (132).

REM sleep disorders and PLMS

REM sleep dysregulation seems to represent a sleep feature of both DM1 (203, 188, 189 201) and DM2 (215, 207, 217, 131, 197). Few polysomnographic studies have dealt with sleep macrostructure in patients with DM1, showing in these patients an increased REM sleep and sleep-onset REM periods (SOREM) (187-189, 191, 201, 203), a decreased in N2 and a trend toward increased N3 (188, 201). A tendency for higher percentage of REM sleep without atonia and REM sleep phasic EMG activity was also noted, even if none of the patients presented clinically assessed REM behaviour disorder (188). It has been suggested that in DM1 there can be a damage in brainstem regions responsible for a global motor disinhibition of REM sleep patterns as reported in narcolepsy.

A recent controlled polysomnographic study showed a high prevalence of REM sleep without atonia (RSWA) also in DM2 patients (207); these authors hypothesized that RSWA may represent a compensatory mechanism against nocturnal respiratory events, and that the brainstem and diencephalon involvement may activate behavioural states during REM sleep (207, 131).

Restless legs syndrome (RLS) and an elevated Periodic Limb Movement (PLM) Index were also described in both DM1 and DM2 patients (188-190, 145, 205-207). It has been suggested that the high number of PLM observed in DM1 patients may be a manifestation of premature ageing of the CNS, in line with the evidence of brain degenerative mechanisms in these patients. In addition, PLM may represent a further puzzling finding for their potential link with an increased autonomic-related cardiovascular risk (191, 338, 339, 340).

COGNITIVE AND BEHAVIOURAL DISORDERS

In DM1 behavioural and cognitive changes are described, even in patients with minimal muscle impairment. Phenotypes associated with CNS involvement can be highly different depending upon the age of disease onset (218, 219). Moreover, it must be taken into account that cognitive impairment in DM patients may also be influenced by other DM symptoms like depression, fatigue and increased daytime sleepiness.

CNS involvement in **congenital** and **childhood** DM1 is more severe. In the congenital form, delayed motor development, behavioural abnormalities, autism, attention deficit hyperactivity disorder (ADHD) and mild to severe mental retardation have been reported (220, 221). Modoni et al. (222) comparing congenital and

classical DM1 showed a global cognitive impairment in congenital types; they hypothesized that a large number of repeats may alter the regulation in brain development, differing from classical DM1.

In the childhood form cognitive and psychiatric disorders are predominant, even in patients with minor motor impairment. IQ and specific cognitive testing evidenced borderline low intelligence and frequent severe visual attention and visual–spatial construction impairments. However, cognitive profiles were heterogeneous, some patients presenting widespread cognitive deficits (intellectual deficit, visual–spatial impairment and attention deficit), whereas others having normal intelligence and visual attention deficit or visual–spatial construction impairment only; patients with severe visual–spatial construction disability had a significantly longer CTG expansion size, as did those with lower IQ. Psychiatric disorders including phobias, anxiety and depression, ADHD were also frequently described in childhood patients (221, 223, 224).

These dysfunctions appear to be due to neurodevelopmental alteration in early life, as longitudinal study showed no further significant decline in cognitive abilities over these ages (225).

The **juvenile form** of DM1 most often manifests at onset with cognitive deficits or psychiatric disorders rather than classic motor manifestations. Douniol et al in their review reported that ADHD, anxiety disorders, phobia, withdrawal and social interactions problems, were the most common psychiatric disorders (226-229). Mean IQ scores were in the borderline range across the different studies, with significant correlations between IQ scores and the CTG repeat number. Speech or language delay could be an isolated manifestation until adolescence (230); however, learning disabilities were the most prominent symptoms reported in juvenile DM1, with deficits in attention, visual spatial/visual constructive skills, verbal working memory, executive functions (226, 228, 230). Woo et al. found a significant decline in all components in the memory domain in juvenile DM1 patients compared to the adult form (231).

In the **adult form** neuropsychological deficits are as variable as muscular symptoms. Global intelligence scores are within the norm, but patients show a worsening in neuropsychological performances and greater cognitive decline than age-matched healthy controls. Cognitive deficits in DM1 patients can be mostly correlated with frontal lobe dysfunction (238, 239) with a selective impairment of executive functioning, attention, visuospatial and visuoconstructive abilities and perceptual reasoning (237, 239, 240), with an apparent saving of verbal skills (147, 240, 241, 214, 242). Low IQ average that decreased with disease duration and the increase of the (CTG)n was described in mild and classic adult-onset DM1; mild DM1 patients showed higher scores in global, verbal and performance intelligence than classic adult-onset forms (147). Other publications about the correlation of CTG-repeat size and neuropsychological deficits showed contradictory results (232-235).

Longitudinal cognitive studies in adult DM1 patients showed a decline in several cognitive domains, mainly in visuospatial and visuoconstructive abilities, memory, attention, and executive functions, with this decline correlating with age at onset and disease duration (238, 244-247), suggesting a possible degenerative brain process (244, 238).

Besides cognitive impairments, behavioural disorders, distinctive personality traits (including avoidant, dependent, hypochondriac, obsessive-compulsive, passive-aggressive, paranoid, schizoid), apathy, social

cognition impairment and mood disorders (depression and anxiety), are frequently reported in DM1 patients, (147, 239, 242, 248-252, 250-251). Moreover, the DM1 population appears to be scarcely concerned about its condition and tends to ignore its symptoms.

Mild cognitive and behavioural symptoms are also present in DM2 patients. In these patients QI and MMSE scores are found to be within the norm, but cognitive deficits similar to those seen in DM1 (altered visuospatial and executive functions, reduced attention and flexibility of thinking), avoidant behavioural trait and depression have been described (218, 243, 7).

The psychological symptoms could be a direct consequence of DM1 pathology affecting the brain, as shown by atypical connectivity in the default mode network (251, 252) or a severe involvement of the dorsolateral prefrontal cortex, cingulum, medial and lateral parietal regions, occipital and temporal lobes, which has been associated with personality changes in many neurological and psychiatric diseases (237). Second hypothesis is that psychological symptoms arise as a consequence of DM1 clinical manifestations, because factors such as pain and fatigue could also bring patients to avoid social situations and eventually to develop avoidant or schizotypal personality traits in response to the disease.

Advanced MRI studies demonstrated across the brain a widespread white matter disruption and a multifocal grey matter volume loss by using various single MRI techniques, including diffusion tensor imaging (DTI) and voxel-based morphometry (VBM), with correlations found between corresponding quantitative MRI parameters and triplet expansion, neuropsychological tests, and the severity of muscular involvement (239, 236-237, 243, 253, 254). Serra et al found significant associations between cortical thickness and patients' social cognition performances, confirming the presence of widespread brain damages associated with cognitive impairment in DM1 patients (252).

However, until now, little is known about changes in CNS causing cognitive deficits and there is wide discussion in literature as to whether CNS dysfunction is caused by altered neurodevelopment, by neuro-dysfunction or by neurodegeneration. The hypothesis of a neurodegenerative disease is endorsed by findings of tau pathology and neurofibrillary degenerations, even if no correlations with CTG-repeat length were found (255). Total-tau increase and Aß42 decrease in cerebral spinal fluid (CSF) have been found in DM1 patients, and central nervous system plasma biomarkers are going to be studied (258, 259). On a molecular basis, MBNL1 and probably CELF may both be involved in CNS alterations, but little is known about molecular defects causing highly variable CNS symptoms in DM1 (256, 257). Overall, CNS dysfunction seems to be multifactorial (7).

There is some evidence that neuropsychological deficits in myotonic dystrophies might partially escape commonly applied neuropsychological test batteries due to still unclear reasons; an international consensus on neuropsychological tests to be generally used in patients with myotonic dystrophy worldwide is strongly recommended. Recent workshops stressed the importance to establish uniform protocols on neuroimaging, neuropsychology and neuropathology to better understand the pattern of involvement of CNS in DM1 patients at time of diagnosis and over time by longitudinal studies, with the aim of clarifying if brain abnormalities are stable or progressive (218, 260). The creation of international DM registry focused on CNS aspects in

order to collect blood, muscular tissue, fibroblasts and cerebrospinal fluid in dedicated biobanks has also been encouraged (218, 219).

NEUROIMAGING FINDINGS

Conventional morphological MRI sequences and voxel-based morphometry (VBM) techniques allowed defining a quantitative analysis of white (WM) and grey matter (GM) loss. VBM studies demonstrated ventricular enlargement and diffuse brain atrophy affecting all lobes, in particular the frontal, parietal lobes, middle and upper temporal gyrus, hippocampus, and subcortical grey matter (GM) (brainstem nuclei, striatum, thalamus, nucleus accumbens, ventral diencephalon and cerebellum) (236, 237, 279-281, 282-285, 241, 243, 277, 286). The functional impact of subcortical volume loss is of particular interest, given the roles of these structures in maintaining wakefulness, regulating sleep architecture, and in cognitive processing (276, 285). Cortical and subcortical GM atrophy seems to be more pronounced in adult-onset compared to juvenile or congenital forms of DM1, while adult DM2 patients showed less profound GM reduction compared to DM1 (236, 237, 275, 243).

Extensive white matter (WM) involvement in all cerebral lobes, cingulum bundle, corpus callosum and brainstem (pons) along middle cerebellar peduncles was also demonstrated; white matter hyperintensities (WML) in DM1 are typically bilateral, asymmetric, and predominantly located in periventricular and subcortical white matter, in frontal and particularly in anterior temporal lobes (ATWML) (240, 235, 236, 243, 286, 283). The structural integrity of WM was mostly evaluated by means of **Diffusion tensor imaging (DTI)** that is a quantitative technique that measures tissue properties, i.e. diffusivity and the directional dependence of microscopic diffusion of water molecules in the brain. Several studies in DM1 patients demonstrated widespread reduction of fractional anisotropy (FA) and increase in mean diffusivity (MD) in association fibres (superior and inferior longitudinal fascicles, inferior fronto-occipital fascicles and uncinate fascicles, limbic system fibre tracts), commissural fibres (mainly corpus callosum) and projection fibres (in the brainstem, internal/external capsules) (261, 236, 237, 241, 283, 287, 288, 253, 289-291). Cabada et al found higher MD in DM1 patients in the thalamus, caudate and putamen confirming previous studies (235, 236, 279, 283) and also in globus pallidus, nucleus accumbens, ventral diencephalon, hippocampus, and amygdala bilaterally (283). Park et al observed a correlation between the grey matter volume in the sensorimotor cortex and alterations in DTI measurements of the corticospinal tract (CST), suggesting that grey matter and white matter abnormalities in DM1 are not independent processes (292).

In general, WM lesions and microstructural white matter changes seem to be more severe and frequent in DM1 than in DM2. These abnormalities seem to be equally pronounced in congenital, juvenile and adult forms of DM1 and were later also confirmed applying a tractography approach (253).

Results of correlation analyses between neuroimaging and clinical parameters are diverse and not well reproducible across studies.

Correlation analysis between neuroradiological alterations and disease duration or age are controversial (236, 288-290). The clinical parameter "disease onset" highly depends on the awareness of patients and their relatives in recognizing disease-related symptoms and most of the previous studies mixed patients with different types of disease onset. Furthermore, early-onset leads to an inevitable interference with normal brain development, so it is difficult to distinguish between pure disease-related brain changes from changes due to impaired brain development. The natural history of GM and WM changes in DM1 and DM2 is largely unclear, and there are only few longitudinal studies analyzing the temporal evolution of brain affection. Some studies suggested a potential progress over time of GM and WM alterations (236, 278, 293, 294, 237, 279, 275, 295).

The largest follow-up series by Cabada et al, after 4 years of follow-up of 24 DM1 patients, described an increase in WML and in ventricular system volume and a decrease in volume of the left thalamus, caudates, putamen, hippocampus and global cortical volume; they also observed a significant increase in MD and decrease in FA for the white matter (293). Conforti et al. included 13 patients in a retrospective follow-up study (mean 13.4 years) and found an increase in WMLs and atrophy over time that was more evident in some families than in others, irrespective of age, disease duration, and CTG repetition (294). Gliem et al., in a 5-year longitudinal 3T-brain MRI follow-up study of a group of middle-aged adult-onset DM1 (and DM-2) revealed only mild progression of GM reduction in DM1 and no significant progression of changes affecting white matter over time, hypothesizing a very slow progression or even a stable course of the disease (278). Some studies observed more pronounced volume reduction of GM in adult-onset DM1 patients than in patients with juvenile (jDM1) and congenital (cDM1) disease-onset (237, 275), while WM alterations did not differ between these group of patients (236, 237, 275), suggesting a strong effect of age on GM alterations. Caso and colleagues (237) argue that the effect of age on GM supports the hypothesis of a degenerative process and that the severe and widespread distribution of WM microstructural damage observed might be interpreted as a result of developmental changes. In line with these results Labayru et al (295) observed that age predicted global GM volume loss and was associated with particular vulnerability to parietal GM regions, suggesting an age-related higher vulnerability of that cortical lobe. 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.

Only few studies investigated correlation of CTG repeat length with GM. Negative correlations between CTG repeat length and GM volumes were described for cingulate gyri, orbitofrontal cortices, frontal poles bilaterally and left pre-central gyrus (287, 295, 296), right temporoparietal junction and precuneus, visual association areas, thalamus, striatum and subcallosal cortex (295). Van der Plas et al, in a cohort of 79 adult DM1 patients, demonstrated that variation in volume of the putamen, thalamus and occipital grey matter volume was negatively related to the size of the CTG repeat expansion (adjusting for covariates), while amygdala volume was positively associated with CTG repeat length (285). Serra et al found significant positive correlation between n(CTG) size and cortical thickness in the left postcentral gyrus and in the left

primary somatosensory cortex; conversely, a significant negative correlation was found between n(CTG) size and cortical thickness in the posterior cingulate cortex bilaterally and in the right lingual gyrus (296). Labayru et al found that areas with atrophies that correlate with increased CTG expansion size were the orbitofrontal area, anterior and posterior cingulate cortex, left sensorimotor areas, right temporoparietal junction and precuneus, visual association areas, thalamus, striatum and subcallosal cortex; moreover, they observed in patients with higher CTG, a decreased FA in the fibres starting in bilateral prefrontal areas, anterior cingulate, temporal cortex, insula and putamen (295). Zanigni et al. found a correlation between triplet expansion size and WM DTI parameters, diffuse to all white matter tracts in supra- and infratentorial compartments as long as patients with very long repeats were not excluded (279). Wozniak et al. reported in their tractography-based study of juvenile/adult-onset DM1 patients an association of CTG repeat length with MD values of the corticospinal tract (CST) and cingulum (254). Park et al (292) observed that CTG repeats were negatively correlated with FA and AD in the posterior limb of the internal capsule (IC) and middle section of the CST, indicating that the white matter microintegrity of these portions of the CST exhibits the greatest susceptibility to genetic factors in patients with DM1.

Correlation analysis between neuroradiological alterations and motor function. Some studies described a correlation between increased muscular impairment, as measured by the MIRS, and areas of atrophy (236, 296, 254, 243, 295). Labayru et al observed a correlation between increased MIRS and atrophy in primary visual and sensorimotor regions, prefrontal ventromedial and orbitofrontal areas, anterior cingulate cortex, precuneus, left thalamus and bilateral striatum (295). Wozniak et al observed a strong association between the level of WM abnormality and MIRS (254). Minnerop et al. (236) observed that reduced FA values in posterior limbs of internal capsules (corticospinal tract) correlated with motor performance in a simple motor task, while reduced FA values along external capsules (which contains corticostriatal projection fibres connecting pre-frontal and temporal areas with basal ganglia, known to play a major role in motion planning and execution) correlated with MIRS. Park et al. (292) described a significant correlation between GM volume loss in the sensorimotor cortex and WM microintegrity alteration of corticospinal tract (CST) (valued with DTI parameters) and motor parameters such as muscle strength, 6 min walking test (6MWT) and handgrip, suggesting a role of corticospinal alteration in motor performance in DM1.

Zanigni et al using the clinical disability scale for DM1 patients (298), observed that the severity of myotonia correlated with a diffuse WM alteration involving also the posterior limb of the internal capsule, the corona radiata adjacent to motor areas and the splenium of corpus callosum; WM differences in the same regions correlated also with the motor domain score of the clinical scale (279).

Correlation analysis between neuroimaging findings and neuropsychological performance. Previous studies analyzed the relationship between neuroimaging and neuropsychological features, with contradictory results. Some studies found significant correlations (299, 237, 241, 243, 283, 292, 254, 295) while others did not (236, 284). However, despite the number of studies published in the literature on this topic, there are not comprehensive enough consensus protocols for neuroimaging and cognitive testing in DM1patients, therefore results between the different studies are difficult to compare (147).

Caso et al. (237), comparing adult (aDM1) and juvenile (jDM1) forms showed, after adjusting for subject's age, a more severe impairment only on the phonemic fluency in aDM. They found that the severity of cognitive deficits correlated with WM damage, but not with GM atrophy; in particular, they observed that MD values of the left corona radiata and association fibres within the frontotemporal WM regions correlated with orientation and attention scores. Schneider-Gold using morphological MRI described in DM1 patients significant correlations between executive functions (namely reduced flexibility of thinking) and atrophy of the left secondary visual cortex; in DM2 these functions correlated with distinct subcortical brain structures and depression was associated with brainstem atrophy (243). Baldanzi et al reported correlations of DTI data with mnesic and visuospatial domains and verbal memory; in particular, they found a negative relationship between left temporal atrophy and verbal memory, between radial diffusivity and delayed verbal memory, visuospatial memory and spatial organization, and a significant negative relationship between axial diffusivity and immediate and delayed verbal memory. Visuospatial impairment was significantly correlated with ventricle enlargement and volume loss in part of the corpus callosum, bilateral cingulated isthmus, right lateral occipital, and pericalcarine cortex; flexibility of thinking correlated with GM volume within left medio-parietal cortex, belonging to the secondary visual cortex (241, 286). Cabada et al. found a significant correlation between visuospatial deficit and a WM major rate lesion, ventricle enlargement and volume loss in the central and anteromedial corpus callosum, bilateral cingulated isthmus, right lateral occipital and right pericalcarine cortex (283). In a more recent follow up study the same authors observed that the worsening in cognitive impairment (working memory and visuospatial skills) was significantly associated with WML load and mean diffusivity increase (293). Labayru et al described 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 region (295).

Wozniak et al, using DTI tractography, found a correlation between white matter integrity in nearly all tracts studied (corticospinal tracts, inferior and superior longitudinal fasciculus, uncinate fasciculus, cingulum, forceps minor) and cognitive functioning, particularly working memory and processing speed (254).

Park et al, using DTI imaging, observed deep grey matter alterations (caudate, pallidum, hippocampus, subthalamic nucleus, thalamus, and substantia nigra) with significant relationship with sleepiness and cognitive functions, particularly attention and executive function (292).

Recently Zanigni et al and Serra et al found correlations of generalized FA reductions with MMSE scores (279, 296). Zanigni et al reported an inverse correlations between the MMSE score and diffusivity changes within the splenium and the posterior part of the corpus callosum, posterior corona radiate, posterior thalamic radiations bilaterally and right retrolenticular part of the internal capsule) (279). Serra et al found significant associations between cortical thickness and patients' social cognition performances, in particular they found a significant positive correlation between correct attribution of sadness and cortical thickness in the left superior temporal gyrus, in the right inferior frontal gyrus, in the right precentral gyrus, in the right angular gyrus, and in the medial frontal gyrus bilaterally; they also showed negative correlations between

performances on the Social Situations Test and cortical thickness in the bilateral precuneus, in the right superior parietal cortex, and in the left lateral temporal and occipital cortex (252).

Correlation analysis between neuroimaging findings and sleep disorders. Only few studies analyzed correlations between sleep disorders and neuroimaging findings in DM. Schneider-Gold, using morphological MRI, described a correlation between daytime sleepiness and volume decrease in the middle cerebellar peduncles, pons/midbrain and the right medio-frontal cortex in DM2 patients but not in DM1 (243). Cabada et al. described an association between daytime hypersomnia and volume loss in the right pallidum and right ventral diencephalon (283). Previous studies observed both regional increases and decreases in hippocampus volume in patients with sleep apnoea and a possible functional link between stimulation of the amygdala and apnoea (329, 330). Van der Plas et al observed larger volume of amygdala and hippocampus, suggesting a possible association with sleep breathing disorders; however, in this study respiratory function was not assessed (285). Wozniak and colleagues using DTI tractography observed that sleepiness was moderately associated with white matter status (MD) in the superior longitudinal fasciculus and cingulum (254).

7. INVOLVEMENT OF OTHER ORGANS AND SYSTEMS

Eyes

Early-onset iridescent cataracts are frequent before 50 years of age in DM patients, observed in about 50–60% of patients, and may be the first and only sign in the mild forms of the disease (9, 262). Slit lamp examination show a multicoloured iridescent appearance located in the posterior lens capsule, findings that are highly suggestive of DM1 or DM2. The mechanisms underlying the pathophysiology of cataract in DM are still largely unknown (9).

New findings of retinal changes by spectral domain optical coherence tomography (OCT) in a cohort of myotonic dystrophy type 1 patients have been described (263). They are consistent with typical retinal pigment epithelium changes and abnormalities of the vitreoretinal interface, particularly epiretinal membranes, resulting in central macular thickness. Both inner and outer retinal alterations are associated with increasing age, suggesting a premature aging of the retina in myotonic dystrophy type 1.

Endocrine abnormalities

Endocrine dysfunctions such as insulin resistance with mild type 2 diabetes, primary hypogonadism (that may produce testicular atrophy, reduced fertility, erectile dysfunction, and low testosterone; women may have a high rate of foetal loss) and severe vitamin D deficiency associated with secondary hyperparathyroidism are frequent in DMs and their occurrence increases with progression of the disease (264-266).

Thyroid dysfunctions are also frequently observed.

Gastrointestinal symptoms

Abnormal liver function tests are common: modest elevations of alanine and aspartate aminotransferase levels (AST e ALT), gammaglutamyltransferase (gamma-GT), and alkaline phosphatase may occur. It is unknown whether these changes represent a primary effect of DM on hepatocytes or a secondary consequence of metabolic derangements, biliary stasis, or fatty liver. The elevation of gamma-GT is suggested to be caused by contractions of bile canaliculi and bile ductules, whereas elevated levels of AST and ALT may have their origin in skeletal muscle involvement, going along with elevations of creatine kinase (CPK) (267, 268, 9).
Intestinal dysmotility with alternating constipation, pseudo-constipation and diarrhoea, reflux and regurgitation are frequently reported symptoms in DM1. Whether these symptoms result from involvement of smooth muscle and striated muscles, enteric neurons, or both, has not been determined (269, 260). Swallowing problems and dysphagia can be caused by myotonia and weakness of the tongue, reduced swallowing reflex and reduced oesophageal motility (271), which causes the major clinical problem by risk of aspiration (9).

Cancer

Epidemiologic studies have confirmed the clinical impression that DM1 is associated with higher risk of cancer, most notably involving the thyroid gland, ovary, colon, endometrium, brain, and eye (choroidal melanoma). A higher incidence for neoplasms was found in several studies (272-274), especially skin cancer (like benign calcifying cutaneous tumours, pilomatricomas), thyroid, testicular, and prostate cancer. Because of the limited number of high-quality surveys and studies about the prevalence of cancer in DM1, further research is needed (9).

OBJECTIVES OF THE STUDY

This is a monocentric prospective study aimed to a better phenotypic and pathogenetic characterization of myotonic syndromes through multidimensional evaluation of cardiological, respiratory and neurological aspects (including both the central nervous system and the peripheral nervous system).

More in detail, the main objectives are:

1. the creation of a computerized database for the collection of clinical and laboratory data;
2. the study of sleep disorders (mainly sleep related breathing disorders and excessive daytime sleepiness) in myotonic syndromes, through the execution of cardiorespiratory monitoring/polysomnographic examinations;
3. the evaluation through skin biopsies of small fibre neuropathy (both somatic and autonomic fibres), receptor dysfunction and ion channels that could be responsible for neuropathic pain;
4. the study of neuropsychological and neuroradiological aspects through MRI.

MATHERIALS AND METHODS

3.1 Patients

Eighty consecutive adult patients with a genetic diagnosis of myotonic syndrome were enrolled at the Neuromuscular Centre of the UOC Neurological Clinic (director: Prof. Liguori) of the Department of Biomedical and Neuromotor Sciences IRCCS Institute of Neurological Sciences, Bologna, during the period from 01/01/2019 to 31/12/2021. The study was approved by the local Ethical Committee.

Among these patients, 5 had a diagnosis of congenital myotonia, 3 patients had a diagnosis of myotonic dystrophy type 2 (DM2) and 72 patients had a diagnosis of myotonic dystrophy type 1 (DM1 or Steinert disease). Because of the small representative sample of patients with DM2 and congenital myotonia, we analyzed only data regarding DM1 patients.

DM1 patients underwent a standardized clinical (neurological, cardiological, respiratory and neuropsychological) and neuroradiological evaluation, being evaluated by a multidisciplinary team of dedicated specialists belonging to the same centre that examined patients during the period of follow-up.

The number of repeats was measured in genomic DNA extracted by peripheral blood leukocyte using Southern blot analysis and DM1 patients were classified according to standard methods into three genetic classes: E1 = 50-150 CTG repeats; E2 = 150-1000 CTG repeats; E3 = more than 1000 CTG repeats.

Depending on age of onset, patients were stratified in three groups: congenital/childhood onset (from birth to 10 years of age); juvenile/adult onset (from 11 to 40 years of age) and late onset (more than 40 years of age).

3.2 Clinical evaluation

Patients enrolled in the study underwent periodic neurological, cardiological and respiratory evaluations on average once a year, during the 3 years of follow-up; we analyzed data at first (T1) and last evaluation (T3).

Neurological examination

Each patient was enrolled in the study during a first neurological evaluation. In addition to neurological physical examination, some questionnaires were administered in order to investigate the grade of disability, the presence of excessive daytime sleepiness, the presence of pain or autonomic symptoms. Patients were always examined by the same neurologists (S. d. P., P. A.).

The severity of muscular involvement was assessed according to the muscular impairment rating scale (MIRS), a validated DM1-specifc rating scale; moreover, a specific disability scale for DM patients was applied, the Neuromuscular Impairment Function and Disability Scale (NIFDS). This scale includes 21 ordinal items divided into four domains: neuropsychological domain (NP), motor domain (MO), myotonia domain (MY) and daily life activity domain (DL). The total score ranges from 0 (normal) to 82 (worst condition). NP includes memory, mood and orientation arranged in five items with a maximum score of 20 points; MO includes nine items with a maximum score of 35 points; MY includes three items with a maximum score of 12 points; DL evaluates the impact of myotonia on the basic life activities with four items and a maximum score of 15 points (298).

The presence of excessive daytime sleepiness (EDS) was assessed by the Italian version of the Epworth Sleepiness Scale (ESS), with scores > 10 considered pathological.

Autonomic functions were tested using the COMPosite Autonomic Symptom Score-31 (COMPASs-31 questionnaire) administered to all patients that underwent skin biopsy and pupillometry.

Cardiological Evaluation

Cardiological assessment included clinical evaluation, basal blood pressure, ECG, 24 h ECG Holter monitoring, and trans-thoracic echocardiogram. In some patients, according to the clinical picture, a cardiac MRI was performed. Conduction abnormalities and arrhythmia on a standard ECG or 24h ECG Holter including PR interval>200 ms, QRS duration>100 ms, atrio-ventricular block, right or left bundle branch block and delayed intraventricular conduction, atrial fibrillation or flutter were considered indicative of cardiac involvement. We measured systolic left ventricular dysfunction as reduction of ejection fraction (EF) below 50%.

Pneumological Evaluation

Respiratory examinations included:

- Arterial blood gas analysis with recording of arterial oxygen partial pressure (PaO2) and arterial carbon dioxide partial pressure (PaCO2); we defined hypercapnia as partial carbon dioxide pressure PaCO2 >45 mmHg and hypoxemia as PaO2 < 80 mmHg.
- Pulmonary function tests (PFTs) with assessment of forced vital capacity (FVC), forced expiratory volume in 1 s (FEV1) maximal inspiratory pressure (MIP) and maximal expiratory pressure (MEP) expressed as percent of predicted value; we defined a restrictive defect when FVC was <80 of the predicted value.

NIV (defined as respiratory support for more than 4 h/daily) included initiation of nocturnal positive airway pressure (NPAP), either continuous (CPAP) or bilevel (BPAP). NIV was started by a pulmonologist in the presence of symptoms suggestive of chronic respiratory insufficiency (dyspnoea at rest, day time hypersomnolence, orthopnea) together with at least one of the following criteria: FVC less than 50% of the predicted value in seated position, apnoea/hypopnoea index greater than 15 events/hour, nocturnal arterial saturation less than 88% for more than 2 cumulative minutes, day time hypercapnia (PaCO2>45 mmHg).

3.3 Sleep recordings

Sleep apnoea was evaluated by means of ambulatory, home-based, cardiorespiratory monitoring; montage was made in the sleep laboratory, in the late afternoon or in the evening, and then patients slept at their homes.

The recording montage included: an oronasal pressure cannula to record airflow, snoring sound, piezoelectric belts to detect thoracic and abdominal respiratory effort, finger pulse oximetry, heart rate, and body position sensors. Data analysis was performed both automatically and visually, in accordance with the criteria established by the American Sleep Disorders Association

In selected cases with respiratory disorders in sleep and /or ESD (assessed by the Epworth scale) a polysomnographic study was performed (simultaneous recording of electroencephalographic, electro-cerebral, surface electromyographic, respiratory - oronasal flow and thoracoabdominal and electrocardiographic movements), in order to identify and characterize changes in sleep (analysis of the macro- and microstructure of sleep and of the respiratory pattern with search for motor disorders). Patients with ESD underwent hypersomnia-specific tests (such as the Multiple Sleep Latency Test - MSLT) and, in the suspicion of secondary narcolepsy, a lumbar puncture to measure the levels of hypocretin.

Respiratory events were scored according to AASM guidelines:

- Obstructive sleep apnoeas were defined as complete cessation of airflow > 10 sec associated with thoracoabdominal movements; central sleep apnoeas were defined by the absence of airflow and thoracoabdominal movements > 10 sec.
- Hypopnoeas were defined as a reduction of airflow between 70% and 10% of baseline values with a desaturation of pulse oximetry by at least 4% from the baseline; the event was considered obstructive if it was related to a flattening of the airflow pressure curve during inspiration, to paradoxical thoracic-abdominal movement or to snoring, and central if it did not meet these requirements.
- Apnoea hypopnoea index (AHI) was defined has the arithmetic mean of the number of apnoeas or hypopnoeas per hour of sleep. An index 5-14.9 was considered mild, 15-30 was moderate, and an index > 30 was severe.
- Oxygen desaturation index (ODI) was defined as the arithmetic mean of desaturations >4% of baseline per hour of sleep (recording time).

3.4 Skin biopsy and pupillometry

Skin byopsy. To visualize somatic and autonomic unmyelinated skin fibres, 3mm punch biopsies were taken from the distal leg and thigh. Specimens were fixed, sectioned, and incubated with primary antibodies. Tissue

sections were processed for immunofluorescence mainly using PGP 9.5 (protein gene product -PGP- a pan-neuronal marker), DbetaH (dopamine b hydroxylase as a marker for noradrenergic fibre of arteriovenous anastomoses (AVAs) and muscle erector pilorum) and VIP (vasoactive intestinal peptide, sudomotor cholinergic fibres of sweat glands) to stain autonomic fibres. Digital images were acquired and studied using a laser scanning confocal microscope. The underlying molecular and functional mechanisms of pain in small fibre neuropathy are still not completely understood. We studied, using primary antibodies (anti-Pan Sodium Channel, used in indirect immunofluorescence associated with pan-neuronal anti-PGP), the expression of sodium ion channel (Nav8) that could be responsible for the neuropathic pain.

Pupillometry: A Ganzfeld ColorDome full-field stimulator (Espion V6, ColorDome Desktop Ganzfeld; Diagnosis LLC, Lowell, MA, United States) was used for the pupillometry test. Both eyes were evaluated separately. All patients underwent a standard ophthalmological examination including optical coherence tomography to evaluate optic nerve retinal nerve fibre layer thickness prior to the test in order to exclude ocular conditions which may interfere with the pupillometric evaluation.

Participants were dark-adapted for 3 min prior to the start of the test. Light stimuli were presented to the tested eye and the pupil responses were recorded from the same eye using the Ganzfeld system equipped with an integrated pupillometer. Stimuli consisted of white light-flashes of 1000 lux and 0.8 s duration presented three times consecutively. The integrated pupillometer system measured the pupil diameter at a 100 Hz sampling frequency. The recording time after stimulus was 15 seconds and the interstimulus interval (ISI) was 30 s. Participants were instructed to keep their eyes open during the duration of the light stimuli as well as following the stimuli. Participants who blinked frequently during the recordings were given another opportunity to repeat the measurements. Pupil traces with excessive artifacts due to long eye blinks or eye closure were excluded from subsequent pupillometric data analysis.

Data were analyzed using custom MATLAB scripts (MathWorks Inc., Natick, MA, USA), one eye for each subject was included in the analysis and it was selected randomly. The calculated pupillometric parameters were: dark-adapted baseline pupil size defined as the median steady-state (baseline) pupil size during the 2 s preceding the light-stimulus onset, and the transient PLR amplitude (peak amplitude) as the difference between the normalized baseline and the minimum normalized PLR after stimulus onset.

3.5 Neuropsychological and psychological assessment

An extensive neuropsychological and psychological assessment was performed in all patients and part of these was also administrated to their caregivers.

Neuropsychological assessment was done using a standardized neuropsychological battery including a general screening test (Mini Mental State Examination MMSE) (353) and a non-verbal test of general intelligence (Raven's Coloured Progressive Matrices CPM '47, Raven, 1940) (354); language skills were assessed using a naming vocabulary test (The Boston Naming Test short 30-items version, Kaplan, 1983; Wendy et al, 1992) (355, 356), an Associative Fluency Test (357) and a Category Words Fluency Test (358).

As concerns memory specific skills, verbal short-term memory span and integrity were assessed using the Digit Span forward test (359) and the immediate recall of Babcock Story Recall Test (BSRT) (360-361), and the auditory verbal learning test immediate recall (RAVLT, Rey, 1960) (362, 371), while visuospatial short-term memory span was investigated using Corsi's Block Test (359). Verbal long-term memory was investigated using the BSRT's delayed recall (361), and the auditory verbal learning test delayed recall (RAVLT) (362, 371), and visuospatial long-term memory was examined by the delayed recall of the Rey-Osterrieth complex figure (ROCF) (362).

Visuospatial and perceptual abilities were investigated by a test measuring visuospatial judgment (BJLOT, Benton et al., 1978) and the Street's completion test (360) measuring apperceptive agnosia. The copy of ROCF further explored the patients' visuoconstructive abilities.

Attention was examined using the Attentional Matrices (360) and SDMT oral version (373).

Regarding executive functions, the assessment consisted in administrating a fast bedside screening test of frontal functions (Frontal Assessment Battery, FAB) (363-364), the Stroop test (362), and the Digit Span Backward (365).

Social cognition was examined with the Eyes mind test (374) and the SET (375).

Levels of self-awareness (or the presence of anosognosia) were measured with the Measurement of Anosognosia Instrument (376) administrated to both patients and caregivers separately.

Psychological assessment of mood and interpersonal behaviours was carried out using the following standardized tests: the presence and severity of depression symptoms was evaluated using Beck Depression Inventory (BDI-II, Beck et al., 1996) and the State-Trait Anxiety Inventory (STAI-Y, Spielberger, 1983) measuring the presence and severity of current symptoms of anxiety and a generalized propensity to be anxious. In order to assess state anger, trait anger, and anger expression and to measure the way these components contribute to medical conditions, the STAXI-II (378) was administrated.

The severity of apathetic symptoms was measured by SAS (377) and an experimental questionnaire (ICS_Intervista sul comportamento spontaneo, Lab. Neuropsicologia geriatrica, Istituto di ricerche farmacologiche Mario Negri, Milano) measuring autonomy in daily activities, interpersonal relationships, and aberrant behaviours, was administrated to the caregivers.

Finally, the Cognitive Reserve Index (CRI) (369) and The Short Form (36) Health Survey (Sf-36)(370) were administered to gather information on intellectual and environmental factors potentially operating on cognitive processes and patient-reported health status respectively.

3.6 Brain MRI acquisition and analysis protocol

Participants underwent a standardized brain MR acquisition protocol using a Siemens MAGNETOM Skyra 3-T (VE11C-SP01) MRI scanner equipped with a high-density head/neck array coil (64 channels).

The MR protocol included high-resolution volumetric T1-weighted images (T1 MPRAGE, magnetization-prepared rapid gradient-echo, sagittal acquisition, isotropic voxel 1x1x1 mm^3, acquisition matrix 256x256,

FOV 256mm, repetition time TR=2300ms, echo time TE= 2.98ms, inversion time TI=900ms, flip angle 9°, GRAPPA acceleration factor 2, total acquisition time 5'21'').

T1-w images were analysed with the pipeline of FreeSurfer (version 6) (https://surfer.nmr.mgh.harvard.edu/), obtaining automatic brain structures segmentation. The volumes of subcortical and cortical regions were considered for both brain sides, with a total of 104 regions. White matter lesion load (resulting as white matter signal hypo-intensities on T1-w images) was also included in the present automatic segmentation.

3.7 Statistical analysis

Patient characteristics were analysed using descriptive statistics. Continuous measurements were presented as means (SD) or as medians (interquartile ranges) and categorical variables as numbers (percentages). No data imputation was made for missing values.

Comparisons between identified groups were performed using t test, Wilcoxon rank-sum test, $\chi 2$ test or Fisher Exact test as appropriate; we also compared continuous variables at baseline vs follow-up using paired t-test or Wilcoxon signed-rank test as appropriate.

Relationships between continuous variables were explored using Spearman correlation coefficients.

Factors that were significantly associated on univariate analysis were then evaluated using multivariate regression (linear for continuous variables and logistic for dichotomous variables), adjusting for age at the visit, sex and disease duration.

Data analysis was conducted with SAS Enterprise Guide version 8.2 (SAS Institute). Statistical significance was set at p-value < 0.05, and all tests were 2-tailed.

Regarding the neuropsychological assessment and brain MRI analysis protocol the normality of data distribution was assessed with Shapiro Wilk test. ANCOVA tests were conducted, to evaluate group effects (E1, E2, Healthy Controls) on brain regions volumes controlling for age, gender and total intracranial volume (added as covariates of no interest). For the significant F tests (after Bonferroni correction for multiple comparisons), post-hoc Tukey's tests were conducted. Raw neuropsychological data were compared between the subgroups (E1 and E2) with ANCOVA tests, controlling for age and education. Correlations between clinical, neuropsychological, and volumetric MR data were evaluated with Pearson's partial correlations controlling for age, gender, education, and total intracranial volume (Benjamini & Hochberg false discovery rate correction for multiple comparisons). Statistical analyses were performed with R software (version 3.5.2).

RESULTS

1. ANAMNESTIC AND NEUROLOGICAL CHARACTERISTICS

Descriptive analysis

We enrolled 80 patients with myotonic syndromes: 5 patients with congenital myotonia (4 patients with chloride channelopathies and 1 patient with sodium channelopathy), 3 patients with DM2 and 72 patients with DM1 (37 males and 35 females). Because of the small representative sample of patients with DM2 and congenital myotonia, we analyzed only data regarding DM1 patients.

Among DM1 patients, 24/72 (33%) were classified as E1 (50-150 CTG repeats), 41/72 (57%) as E2 (150-1000 CTG repeats) and 7/72 (9.7%) patients were classified as E3 (more than 1000 CTG repeats).

In 25/72 (34.7%) patient disease onset was in adulthood, in 18/72 (25%) patient onset was in late adulthood, 15/72 (20%) patients had a juvenile onset and 14/72 (19.4%) patients had a congenital/childhood onset.

In the group "congenital DM1" there was a predominance of E3 expansion (71.4% of patients), while no cases of E1 expansion were found; in the group "juvenile DM1" the E2 expansion predominated (29.3%); in the "adult" and "late onset" group there was a predominance of E1 expansion size (Table 1). There were no significant differences in terms of sex between the 3 classes (Table 1).

Almost all patients with E3 expansion size reported a maternal transmission (6/7 patients, 86%); paternal transmission prevailed among the E2 group (26/41 patients, 63.4%), while for the majority of E1 patients the way of transmission was unknown (13/24 patients, 54.17%) (Table 2).

Median age at onset was at birth for E3 patients, 20 (13-30) years for E2 patients, 36 (25-47.5) years for E1 patients. Muscle weakness was present from birth in the E3 class, while in E2 and E1 classes median age at onset of hyposthenia was at 27 (15-43) and 39 (30-51) years respectively. Myotonia age of onset was reported at 10 (6-25), 24 (15-35) and 35 (25-47) years in E3, E2 and E1 patients respectively.

Perinatal suffering, delay in psychomotor development and learning problems were reported more frequently in the E3 class compared to the other two classes of expansion (Table 1).

Among extra-muscular symptoms at onset, excessive daytime sleepiness was present in 19.7% of patients, fatigue in 28.17% and palpitations in 4.3% of patients (considering the whole group of patients).

At first neurological evaluation (T1) the grade of muscular involvement evaluated with the Muscular Impairment Rating Scale (MIRS) showed proximal as well as distal limb weakness (MIRS score = 4) in class E2 and E3 patients, while E1 patients showed a lower degree of muscle involvement, mainly of distal limbs (MIRS score = 3). The presence of dysphagia and myopathic facies was more frequent in E3 class patients (Table 4).

Median disease duration at first evaluation was 29.9, 17.3 and 11.5 years respectively in E3, E2 and E1 classes (Table 1).

Correlation analysis

Analyzing the possible correlations among the anamnestic/neurological parameters and the class of expansion, we found that larger expansion size of CTG were significantly correlated with maternal transmission (p = 0.000), earlier age at onset (p=0.0002), presence of weakness at birth (p=0.0035), perinatal suffering (p = 0.002), delay in psychomotor development and difficulties in learning (p= 0.000); moreover, the size of triplet repeat expansion showed a significant correlation with the involvement of the facial musculature both at first and at last examination (p= 0.0066 and p =0.019 respectively) (Table 2).

Stratifying by age of onset (congenital/childhood onset vs juvenile/adult onset vs late onset) we observed that congenital/childhood onset were significantly correlated with maternal transmission (p= 0.000), perinatal suffering, delay in psychomotor development and learning problems (p= 0.000); age at onset of myotonia and muscular weakness were significantly lower and disease duration longer in this group of patients (p= 0.001) (Table 3).

We found a significant correlation between the grade of disability (measured both through the MIRS score and the Neuromuscular impairment function and disability scale-NIFDS) and disease duration, for both the scores (Table 6) (p= 0.0001).

Table 1. CTG expansion size and age at onset

	Total (%)	**Congenital** (0- 1 month)/ **Childhood** (1-10 years)	**Juvenile** (10–20 years)	**Adult DM1** (21-40 years)	**Late onset** (>40 years)	**Sex F N (%)**	**Sex M (N %)**
E1	24 (33.3)	0 (0.0)	3 (12.5)	12 (50.0)	9 (37.5)	12 (50.00)	12 (50.00)
E2	41 (57.0)	9 (21.9)	12 (29.3)	12 (29.3)	8 (19.5)	22 (53.66)	19 (46.34)
E3	7 (9.7)	5 (71.4)	0 (0.0)	1 (14.3)	1 (14.3)	1 (14.29)	6 (85.71)
Total (%)	72	14 (19.4)	15 (20%)	25 (34.7)	18 (25%)	35	37

E1 = 50–150 CTG repeats; E2 = 150–1000 CTG repeats; E3 = more than 1000 CTG repeats

Table 2. Anamnestic/neurological characteristics and class of CTG expansion

Total of patients		**E1 n (%) 24 (100)**	**E2 n (%) 41 (100)**	**E3 n (%) 7 (100)**	**Total n (%) 72 (100)**	**P**
Transmission	Maternal	3 (12.5)	9 (21.95)	6 (85.7)	18 (25)	**0.000**
	Paternal	8 (33.3)	26 (63.4)	0 (0)	34 (47.22)	
	Unknown	13 (54.17)	6 (14.63)	1 (14.29)	20 (27.78)	
Perinatal suffering		1 (4.17)	2 (4.88)	4 (57.14)	7 (9.72)	**0.002**
Reason for diagnosis	Onset of symptoms	10 (41.67)	30 (73.17)	7 (100)	47 (65.28)	**0.032**
	Birth of affected child	8 (33.33)	5 (12.20)	0 (0)	13 (18.06)	
	Family screening	6 (25)	6 (14.63)	0 (0)	12 (16.67)	
Delay in psychomotor development		0 (0)	6 (14.63)	5 (71.43)	11 (15.28)	**0.000**
Learning problems		1 (4.17)	10 (24.39)	6 (85.71)	17 (23.61)	**0.000**
Muscular weakness at onset						-
Excessive daytime sleepiness at onset		5 (20.83)	9 (22.5)	0 (0)	14 (19.72)	-
Fatigue at onset		5 (20.83)	14 (35)	1 (14.29)	20 (28.17)	-
Palpitations at onset		3 (24)	0 (0)	0 (0)	3 (4.23)	-
Site of onset myotonia	Distal upper limbs	19 (79.17)	35 (85.37)	6 (85.71)	60 (83.33)	-
	Lower limbs	0 (0)	1 (2.44)	0 (0)	1 (1.39)	
	Multi-district interest	2 (8.33)	5 (12.2)	1 (14.29)	8 (11.11)	
Site of onset hyposthenia	Distal upper limbs	12 (52.17)	23 (56.10)	2 (28.57)	37 (52.11)	-
	Distal lower limbs	8 (34.78)	11 (26.83)	1 (14.29)	20 (28.17)	
	Proximal lower limbs	0 (0)	1 (2.44)	0 (0)	1 (1.41)	
	Multi-district interest	1 (4.35	6 (14.63)	4 (57.14)	11 (15.49)	
Age at onset - Median (IQR)		36 (25-47.5)	20 (13-30)	0 (0-25)	25 (15-41)	**0.0002**
Age at first evaluation (T1) Median (IQR)		48.3(41.2-53.9)	44.1(30.6-48.6)	30.4 (27-49)	45.4(32.9-50.5)	**0.0562**
Age at onset myotonia Median (IQR)		35 (25-47)	24 (15-35)	10 (6-25)	25 (16-40)	**0.0032**
Age at onset muscle weakness Median (IQR)		39 (30-51)	27 (15-43)	0 (0-39)	29.5(18.5-45.5)	**0.0035**
Disease duration at T1 Median (IQR)		8.8 (3-20.4)	16.9 (8-25.8)	27.1 (24-33.4)		

Table 3. Anamnestic/neurological characteristics and age at onset

Total of patients		**Late onset n (%) 18 (100)**	**Juvenile/Adult n (%) 40 (100)**	**Congenital/ Childhood n (%) 14 (100)**	**Total n (%) 72 (100)**	**P**
Transmission	Maternal	2 (11.11)	5 (12.5)	11 (78.57)	18 (25)	**0.000**
	Paternal	8 (44.4)	23 (57.5)	3 (21.43)	34 (47.22)	
	Unknown	8 (44.4)	12 (30)	0 (0)	20 (27.78)	
Perinatal suffering		0 (0)	1 (2.5)	6 (42.86)		**0.000**
Delay in psychomotor development		0 (0)	0 (0)	11 (78.57)	11 (15.28)	**0.000**
Learning problems		0 (0)	4 (10)	13 (92.86)	17 (23.61)	**0.000**
Age at first evaluation (T1) Median (IQR)		57 (53-63)	45.5 (38-53)	33 (25-36)	49.5 (36-55.5	**0.0001**
Age at onset myotonia Median (IQR)		48 (46-53)	25 (18.5-29.5)	6.5 (5-12)	25 (16-40)	**0.0001**
Age at onset muscle weakness Median (IQR)		52 (47-54)	27 (23-36)	4.5 (0-12)	29.5(18.5-45.5)	**0.0001**
Disease duration Median (IQR)		7 (4-13)	20 (14-27.5)	29.5 (20-36)	19.5 (10.5-27.5)	**0.0001**

The table shows only the items with significant correlations

Congenital/childhood onset= from birth to 10 years; Juvenile/adult onset = 11-40 years; Late onset = > 40 years.

Table 4. Neurological Examination at first evaluation (T1) and class of CTG expansion

	E1 n (%) **18 (100)**	**E2 n (%)** **40 (100)**	**E3 n (%)** **14 (100)**	**Total** **72 (100)**	**P**
Grip myotonia	20 (87)	36 (100)	6 (100)	62 (95.4)	-
Dysarthria	12 (52.2)	23 (62.2)	6 (100)	41 (62.1)	-
Dysphagia	5 (21.7)	10 (27.8)	2 (33.3)	17 (26.2)	-
Myopathic face	13 (56.5)	32 (88.9)	6 (100)	51 (78.5)	**0.0066**
	E1 median (IQR)	**E2 median (IQR)**	**E3 median (IQR)**	**Total**	
MIRS	3 (2-4)	4 (3-4)	4 (3-4)	3 (3-4)	**0.0324**

Table 5. Neurological Examination at last evaluation (T3) and class of CTG expansion

Total	**E1 n (%)** **24**	**E2 n (%)** **41**	**E3 n (%)** **6**	**Total** **71**	**P**
Grip myotonia	20 (83.33)	38 (92.68)	6 (100)	64 (90.14)	**0.033**
Tongue myotonia	13 (54.17)	31 (77.5)	6 (100)	50 (71.43)	
Dysarthria	15 (62.5)	30 (73.17)	6 (100)	51 (71.83)	-
Dysphagia	11 (45.83)	25 (65.79)	3 (50)	39 (57.35)	-
Myopathic face	16 (66.67)	38 (92.68)	6 (100)	60 (84.51)	**0.019**
	E1 median (IQR)	**E2 median (IQR)**	**E3 Median (IQR)**	**Total**	**p**
MIRS	3 (3-4)	4 (3-4)	4 (4-4)	4 (3-4)	**0.0212**

Table 6. Disease duration and grade of disability

Disability score	**P value**
MIRS	**<.0001**
NIFDS Total score	**<.0001**
NP_total	0.0051
MO_total	<.0001
My_total	0.0004
DL_total	<.0001

NIFDS = "Neuromuscular impairment function and disability scale" and its subitems: neuropsychological domain (NP), motor domain (MO), myotonia domain (MY) and daily life activity domain (DL).

MIRS = "Muscular impairment rating scale" (see the methods section).

2. CARDIOLOGICAL EVALUATION

Descriptive analysis

At the time of the first visit (T1) we found a cardiological involvement in 16/23 (69.5%) E1, in 27/39 (69.2%) E2, and in 6/7 (85.7%) E3 patients, with a prevalence of conduction disorders within all expansion classes (Table 7). After 3 years of follow-up (T3) 5 patients belonging to E1 class showed progression in cardiac involvement with development of more than one disorder; among the E2 group 3 patients developed a cardiomiopathy and 1 patient had a progression with the development of more than one disorder; among the E3 group, 1 patient developed a cardiomiopathy (Table 8).

Six patients had prophylactic pace-maker (PM) implantation (1 E1 patients, 4 E2 patients and 1 E3 patient) (Table 17).

Correlation analysis

Comparing the 3 genetic classes we found that patients belonging to E3 class of expansion had greater cardiological involvement on the Holter ECG at the time of the first evaluation (Table 11). No differences among the different classes were found regarding ECG or echocardiography abnormalities, cardiological symptoms, pace-maker implantation both at the time of the first and the last evaluation (Table 9, 10, 12-17). Similarly, stratifying patients by age of onset (congenital/childhood onset vs juvenile/adult onset vs late onset) we did not find significant differences among the three groups of patients.

Comparing continuous cardiological parameters (PR and QRS interval length, mean heart rate -HR-, min HR, max HR, ejection fraction) among the 3 genetic classes at T1 and T3, we observed a significant decrease of mean HR in E2 class of expansion (p=0.035) (Table 18). Analyzing the whole sample of patients we found a statistically significant increase of PR interval (p = 0.0106) at T3 (Table 19).

Disease duration was associated with longer PR length on the ECG (p= 0.0036) and lower Ejection Fraction on the echocardiography (p= 0.009) (Table 20).

In order to analyze the interplay between neurological, respiratory and cardiac involvement, we compared different groups of patients at T3 analyzing all the continuous parameters registered (age at onset, disease duration, grade of disability measured through MIRS and NFDS score, BMI, blood gas analysis values, respiratory function test parameters, cardiological variables, Epworth Score and sleep parameters).

Comparing patients with cardiopathy (defined as ejection fraction ≤ 50%) **and without cardiopathy** (ejection fraction >50%) we did not find a statistically significant difference among the 3 classes of expansion (Table 21) nor between males and females. We observed that patients with cardiopathy had a longer disease duration (p=0.0410), a higher BMI (0.0169), lower values of CVF and FEV1 (p = 0.0056 and p = 0.0322 respectively), lower HR max (p = 0.0123), higher obstructive apnoea/hypopnoea and mixed apnoea index (p = 0.0256) (Table 22).

Analyzing possible relationships between continuous variables using Spearman correlation coefficients we found that Ejection Fraction had a positive association with CVF (p=0.026) and FEV1(p=0.038), while a negative association was observed with BMI (p= 0.002), Motor and Myotonia domain of the NIFDS scale (p=0.049 and p=0.0307) (Table A).

In the adjusted logistic regression, higher CVF values were significant predictors for a reduced risk of cardiopathy (OR=0.92, CI95% [0.86-0.99], p value 0.025), while FEV1 and obstructive apnoea/hypopnoea and mixed apnoea index were no longer associated with cardiopathy.

Comparing patients with conduction disorder (defined as PR ≥200 msec, QRS ≥100 msec) **and without conduction disorder** we did not find a statistically significant difference among the 3 classes of expansion (Table 23) nor between males and females. We found that the first group of patients had longer disease duration (p =0.0158), higher MIRS score values (p=0.0339), lower ejection fraction (p=0.0475), higher ODI index (p=0.0156), obstructive apnoea/hypopnoea and mixed apnoea index (p= 0.0039) and AHI index (p=0.0023) and lower nocturnal saturation values (minimal and mean SaO2, p=0.0651 and p=0.0115 respectively) (Table 24).

Applying logistic regression we found that the association with ejection fraction, ODI, obstructive apnoea/hypopnea and mixed apnoea index and nocturnal saturation values were no longer significant.

Using Spearman correlation coefficients between continuous variables, we found that:

- PR interval length had a positive association with MIRS (p=0.0002), daily life activity domain of the NIFDS scale (p=0.0136), oxygen desaturation index (p =0.0147) and obstructive apnoea/hypopnoea and mixed apnoea index (p=0.0037)
- QRS interval length had a positive association with ODI index (p=0.022) and obstructive apnoea/hypopnoea and mixed apnoea index (p=0.0031)

Table 7. Cardiac involvement at first evaluation (T1)

	E1 n (%)	E2 n (%)	E3 n (%)	Total	p
No alterations	7 (30.43)	12 (30.77)	1 (14.29)	20 (28.99)	
Conduction abnormalities	8 (34.78)	17 (43.59)	4 (57.14)	29 (42.03)	
Cardiomyopathy	1 (4.35)	1 (2.56)	0 (0)	2 (2.9)	-
Ischaemic heart disease	1 (4.35)	0 (0)	0 (0)	1 (1.45)	
Atrial Fibrillation/Flutter	2 (8.7)	0 (0)	0 (0)	2 (2.9)	
More than one disorder	4 (17.39)	9 (23.08)	2 (28.57)	15 (21.74)	
Total of patients	23 (100)	39(100)	7(100)	69(100)	

Table 8. Cardiac involvement at last evaluation (T3)

	E1 n (%)	E2 n (%)	E3 n (%)	Total	p
No alterations	6 (26.09)	11 (28.21)	0 (0)	17 (25)	
Conduction abnormalities	6 (26.09)	14 (35.9)	4 (66.67)	24 (35.29)	
Cardiomyopathy	1 (4.35)	4 (10.26)	1 (16.67)	6 (8.82)	-
Ischaemic heart disease	1 (4.35)	0 (0)	0 (0)	1 (1.45)	
Atrial Fibrillation/Flutter	1 (4.35)	0 (0)	0 (0)	1 (1.47)	
More than one disorder	9 (39.13)	10 (25.64)	1 (16.67)	20 (29.41)	
Total of patients	23 (100)	39(100)	6(100)	68(100)	

Table 9. ECG – first evaluation (T1)

	E1 n (%)	E2 n (%)	E3 n (%)	Total	p
Normal	12 (50)	13 (32.5)	1 (14.29)	26 (36.62)	
Atrio-ventricular block	3 (12.5)	6 (15.0)	0 (0)	9 (12.68)	
Right or left bundle branch block / Delayed intraventricular conduction	5 (20.83)	13 (32.5)	4 (57.14)	22 (30.99)	-
Atrial fibrillation /Flutter	1 (4.17)	1 (2.5)	1 (14.29)	3 (4.23)	
More than one disorder	3 (12.5)	7 (17.5)	1 (14.29)	11 (15.49)	
Total of patients	24 (100)	40 (100)	7 (100)	71 (100)	

Table 10. ECG – last evaluation (T3)

	E1 n (%)	E2 n (%)	E3 n (%)	Total	p
Normal	9 (39.13)	13 (33.33)	1 (16.67)	23 (33.82)	
Atrio-ventricular block	4 (17.39)	4 (10.26)	2 (33.33)	10 (14.71)	
Right or left bundle branch block / Delayed intraventricular conduction	5 (21.74)	8 (20.51)	2 (33.33)	15 (22.06)	-
Atrial fibrillation /Flutter	1 (4.35)	0 (0)	0 (0)	1 (1.47)	
More than one disorder	4 (17.39)	14 (35.90)	1 (16.67)	19 (27.94)	
Total of patients	23 (100)	39 (100)	6 (100)	68 (100)	

Table 11. Holter ECG- first evaluation (T1)

	E1 n (%)	E2 n (%)	E3 n (%)	Total	p
Normal	13 (68.42)	25 (83.33)	4 (57.14)	42 (75)	
Atrio-ventricular block	3 (15.79)	0 (0)	0 (0)	3 (5.36)	
Right or left bundle branch block / Delayed intraventricular conduction	1 (5.26)	4 (13.33)	2 (28.57)	7 (12.5)	**0.035**
Atrial fibrillation /Flutter	2 (10.53)	0 (0)	0 (0)	2 (3.57)	
More than one disorder	0 (0)	1 (3.33)	1 (14.29)	2 (3.57)	
Total of patients	19 (100)	30 (100)	7 (100)	56 (100)	

Table 12. Holter ECG – last evaluation (T3)

	E1 n (%)	E2 n (%)	E3 n (%)	Total	p
Normal	14 (73.68)	28 (80)	4 (66.67)	46 (76.67)	
Atrio-ventricular block	3 (15.79)	1 (2.86)	2 (33.33)	6 (10)	
Right or left bundle branch block / Delayed intraventricular conduction	1 (5.26)	4 (11.43)	0 (0)	5 (8.33)	-
Atrial fibrillation /Flutter	0 (0)	1 (2.86)	0 (0)	1 (1.67)	
More than one disorder	1 (5.26)	1 (2.86)	0 (0)	2 (3.33)	
Total of patients	19 (100)	35 (100)	6 (100)	60 (100)	

Table 13. Echocardiogram – first evaluation (T1)

	E1 n (%)	E2 n (%)	E3 n (%)	Total	p
Normal	18 (75)	30 (83.33)	5 (71.43)	53 (79.10)	
Hypertrophy	0 (0)	0 (0)	1 (14.29)	1 (1.49)	
Hypokinesia/systolic ventricular dysfunction	5 (20.83)	6 (16.67)	1 (14.29)	12 (17.91)	
Severe valvulopathy	1 (4.17)	0 (0.0)	0 (0.0)	1 (1.49)	-
Total of patients	24 (100)	36 (100)	7 (100)	67 (100)	

Table 14. Echocardiogram – last evaluation (T3)

	E1 n (%)	E2 n (%)	E3 n (%)	Total	p
Normal	13 (59.09)	26 (68.42)	4 (66.67)	43 (65.15)	
Hypertrophy	5 (22.73)	2 (5.26)	2 (33.33)	9 (13.64)	-
Hypokinesia/systolic dysfunction	4 (18.18)	10 (26.32)	0 (0)	14 (21.21)	
Total of patients	22 (100)	38 (100)	6 (100)	66 (100)	

Table 15. Echocardiogram: Ejection Fraction (T1 and T3)

	E1 median (IQR)	E2 median (IQR)	E3 median (IQR)	Total	P
Ejection Fraction – T1	62.4 (58-66.7)	60 (55.35-66.1)	63.4 (55-76)	61 (55.7-66.7)	-
Ejection Fraction – T3	61 (57-63.4)	60 (55-65)	61 (60-69)	60.7 (55.8-65)	-

Table 16. Symptoms at last evaluation (T3)

	E1 n (%)	E2 n (%)	E3 n (%)	Total	p
No symptoms	21 (91.3)	37 (94.87)	6 (100)	64 (94.12)	-
Palpitations	1 (4.35)	1 (2.56)	0 (0)	2 (2.94)	
Exertional dyspnoea	1 (4.35)	0 (0)	0 (0)	1 (1.47)	
Reduced stress tolerance	0 (0)	1 (2.56)	0 (0)	1 (1.47)	
Total of patients	23 (100)	39 (100)	6 (100)	68 (100)	

Table 17. Pacemaker (PM) implantation

	E1 n (%)	E2 n (%)	E3 n (%)	Total	p
Yes	1 (4.17)	4 (9.76)	1 (14.29)	6 (8.33)	-
No	23 (95.83)	37 (90.24)	6 (85.71)	66 (91.67)	-

Table 18. Cardiological parameters at T1 and T3 in the different classes of expansion

	First evaluation (T1)	Last evaluation (T3)	P-value
Variable	**Median (IQR)**	**Median (IQR)**	
E1 (N=24)			
PR interval length	184 (164-200)	175 (163-225)	-
QRS interval length	109.5 (101-119)	113.5 (99-141)	
Heart rate (HR) mean	62 (58-70)	63.5 (59-69)	
HR min	41 (35-43)	41 (33-43)	
HR max	111 (97-117)	114.5 (100-127)	
Ejection Fraction	62.4 (58-66.7)	61 (57-63.4)	
E2 (N=41)			
PR interval length	191 (165-210)	192.5 (180-220)	0.053
QRS interval length	116 (100-122)	112 (100-133)	-
Heart rate (HR) mean	74 (70-80)	71 (64-79)	**0.0153**
HR min	45 (40-52)	44 (39-46)	0.0554
HR max	126 (120-134)	125 (116-135)	-
Ejection Fraction	60 (55.4-66.1)	60 (55-65)	-
E3 (N=7)			
PR interval length	181.5 (178-203)	181 (181-270)	-
QRS interval length	114.5 (109-137)	105 (102-171)	
Heart rate (HR) mean	68.5 (59-73)	67 (58.5-75.5)	
HR min	42.5 (41-46.5)	42 (41-44)	
HR max	117 (106-132)	113 (93-127)	
Ejection Fraction	63.4 (55-76)	61	

Table 19. Cardiological parameters at T1 and T3 – total of patients

	First evaluation (T1)	Last evaluation (T3)	
	Median (IQR)	**Median (IQR)**	**p-value**
Mean HR	71 (63-76)	69 (61.5-74.5)	-
PR interval length	**187** (165-205)	**188** (168-220.5)	**0.0106**
QRS interval length	113 (102-122)	112.5 (101-133.5)	-
Ejection Fraction	61.5 (55.7-66.7)	60.7 (55.8-65)	-

Table 20. Table Disease duration and cardiological parameters at T3

Cardiological parameters	**P-value**
Mean HR	-
Min HR	-
Max HR	-
PR	**0.0036**
QRS	-
Ejection Fraction	**0.009**

Table 21. Distribution of patients with and without cardiopathy among the 3 classes of expansion at T3

	E1 n (%)	**E2 n (%)**	**E3 n (%)**	**Total**	**p**
Without cardiopathy	17 (32.69)	31 (59.62)	4 (7.69)	52	-
With cardiopathy	2 (22.22)	6 (66.67)	1 (11.11)	9	
Total	**19**	**37**	**5**	**61**	

Table 22. Comparison between patients with and without cardiopathy at last evaluation (T3)

	Without cardiopathy (FE >50%)	**With cardiopathy (FE <50%)**	**P-value**
Total of patients n (%)	**52 (100)**	**9 (100)**	
Females n (%)	30/52 (57.7%)	2/9 (22%)	**-**
	Median (IQR)	**Median (IQR)**	
Age at onset	24 (13.5-36)	24 (20-30)	-
Age at evaluation	47.2 (34.1-53.2)	51.8 (40.9-56.7)	-
Disease duration	**17 (10.1-27.5)**	**26.7 (20.7-31.1)**	**0.0410**
MIRS	3 (3-4)	4 (3.5-4)	-
NP_total	3 (1-6)	1 (1-7)	-
MO_total	12 (7-15)	14 (9-17)	-
My_total	6 (5-8)	7 (6-10)	-
DL_total	2 (1-5)	4 (2-5)	-
NIFDS total	24 (14-30)	25 (19-39)	-
Epworth	8 (6-10)	7 (4-10)	-
BMI	**23.1 (21-25.5)**	**25.9 (23.9-30.2)**	**0.0169**
PCO2	46 (41-48.2)	44 (42-46)	-
pO2	87.5 (80-93)	79 (77-85)	-
SaO2	97 (96-97.5)	96.4 (95-97)	-
CVF	**80 (65-95)**	**56 (41-74)**	**0.0056**
FEV1_	**81 (64-93)**	**55 (43-78)**	**0.0322**
MIP	33.5 (25-47)	36 (24-53)	-
MEP	28 (22-37)	16 (13-25.5)	-
HR mean media	69 (61-76)	69 (64-72)	-
HR min	43 (37-45)	44 (41-46)	-
HR max	**126 (115-135)**	**107.5 (101-114)**	**0.0123**
PR interval length	188 (163-221)	211 (196.5-220)	-
QRS interval length	111 (102-132)	170.5 (131.5-183)	-
Oxygen desaturation index (ODI)	11.37 (3-20.35)	17.95 (11.1-27.3)	-
Min SaO2 (nocturnal)	82 (77-89)	80 (75-85)	-
Mean SaO2(nocturnal)	94 (91.5-94.7)	91 (88.2-93)	-
Obstructive apnoea/hypopnoea index and mixed apnoea	**10.6 (3.4-17.2)**	**34 (16-39)**	**0.0256**
Central apnoea index, n/h	0.7 (0-2.16)	8.6 (0-17.2)	-
Apnoea/Hypopnoea Index (AHI)	10.93 (4-17.94)	25 (16-34)	-

Table 23. Distribution of patients with and without conduction disorder among the 3 classes of expansion

	E1 n (%)	**E2 n (%)**	**E3 n (%)**	**Total**	**p**
Without conduction disorder	13 (41.94)	16 (51.61)	2 (6.45)	31	-
Conduction disorder (PR ≥200 msec, QRS ≥100 msec)	5 (29.41)	11 (64.71)	1 (5.88)	17	
Total	**18**	**27**	**3**	**48**	

Table 24. Comparison between patients with and without conduction disorder (T3)

	Without conduction disorder	**Conduction disorder (PR ≥200 msec, QRS ≥100 msec)**	p
Total of patients n (%)	31 (100)	17 (100)	-
Females n (%)	18 (56.1%)	8/17 (47.1%)	**-**
	Median (IQR)	**Median (IQR)**	-
Age at onset	27 (15-42)	21 (13-35)	-
Age at evaluation	46.94 (31.78-52.96)	51.98 (45.41-55.32)	-
Disease duration	**14.51 (7.45-25.9)**	**26.89 (16.98-35.39)**	**0.0158**
MIRS	**3 (3-4)**	**4 (3-4)**	**0.0339**
NP_total	2 (1-6)	2.5 (1-6)	-
MO_total	11 (6-14)	14 (9-16)	-
My_total	6 (4-7)	6 (5-8)	-
DL_total	2 (0-4)	5 (2-5)	-
NIFDS total	22 (12-29)	28.5 (22-33)	-
Epworth	8.5 (7-11)	8 (5-9)	-
BMI	23.2 (20.8-25.3)	23.9 (22.45-26.55)	-
PCO2	46 (42-48)	45 (39.5-48)	-
pO2	86.2 (80-94)	88 (83-92)	-
SaO2	97 (96-98)	97 (96-97.45)	-
CVF	85 (63-99)	79 (66.5-88)	-
FEV1	82 (64-97)	73.35 (64-82)	-
MIP	35 (23-51.5)	33 (28-36)	-
MEP	31 (21-39)	26 (23-34)	-
HR mean media	66 (60-79)	68 (62-71)	-
HR min	42 (37-44.5)	41.5 (35-44.5)	-
HR max	125 (114-134)	118 (103-140.5)	-
Ejection Fraction	**61.45 (60-65)**	**60 (55-61.9)**	**0.0475**
Oxygen desaturation index (ODI)	**5 (1.4-21)**	**16.7 (14.6-22.1)**	**0.0156**
Min SaO2 (nocturnal)	**86 (79-89)**	**80 (75-83)**	**0.0651**
Mean SaO2(nocturnal)	**94 (92-95.2)**	**91 (87.9-92.8)**	**0.0115**
Obstructive apnoea/hypopnoea index + mixed apnoea	**4.9 (1.3-11.9)**	**17.3 (11.5-19.7)**	**0.0039**
Central apnoea index, n/h	0.2 (0-2.16)	0.8 (0.2-3.1)	0.4001
Apnoea/Hypopnoea Index (AHI)	5 (2.15-13.2)	17.94 (16-23.87)	**0.0023**

3. RESPIRATORY EVALUATION

Descriptive analysis

- **Arterial blood gas analysis**

At the time of the first visit (T1) 62 patients underwent arterial blood gas analysis.

Hypoxemia and/or hypercapnia were found in 9/23 (39%) E1, 18/34 (52.9%) E2 and 2/5 (40%) patients with E3 class of expansion (Table 25).

At the time of the last visit (T3) these abnormalities were observed respectively in 7/19 (36.8%) E1, 18/32 (56.25) E2 and 0/5 E3 patients (Table 26).

- **Respiratory function test**

At the time of the firs visit (T1) respiratory function test were performed on 71 patients. A restrictive syndrome was observed in 7/24 (29%) E1 patients, 29/40 (72%) E2 patients and 7/7 (100%) E3 patients (Table 27).

At the time of the last visit (T3) a restrictive syndrome was found in 10/22 (45.55%) E1, 22 /34 (64.7%) E2 and 5/6 (83.3%) E3 patients (Table 28).

Between T1 and T3 Non Invasive Ventilation (NIV) was started whit good compliance by 2 patients with E2 class of expansion and 2 E1 patients; 2 other patients (1 E1 and 1 E2) had to start NIV but refused.

For other 25 patients (6 E1, 15 E2 and 4 E3 patients) NIV was indicated before T1, but only 7 of them declared good compliance with NIV.

Correlation analysis

Analyzing the continuous pulmonary parameters in the different **genetic classes** we found that forced vital capacity (FVC), forced expiratory volume (FEV1), maximal inspiratory pressure (MIP) and maximal expiratory pressure (MEP) were significantly reduced in patients with E3 class of expansion compared to the other genetic classes ($p = 0.0003$, $p = 0.0020$, $p = 0.0178$, $p = 0.035$ respectively) at first evaluation (T1); these results were confirmed at last evaluation (T3), with the exception of MIP (Table 29, 30).

On the other hand, arterial oxygen partial pressure (PaO_2) and arterial carbon dioxide partial pressure ($PaCO_2$) values showed no significant differences among the 3 groups of patients, both at T1 and T3 (29, 30).

Similar results were obtained comparing patients with **different age at onset** (congenital/childhood onset vs juvenile/adult onset vs late onset) (Tables 31 and 32).

Analyzing continuous pulmonary parameters at **T1 and T3** we observed a significant decrease of FEV1 and an unexpected increment of MIP in the E2 group of patients ($p=0.0066$ and $p = 0.0054$ respectively) (Table 33); studying the whole sample of patients at T1 and T3 we found a statistically significant decrease of FEV1 ($p=0.0033$), increment in MIRS score ($p=0.0232$) and an unexpected increment of MIP and MEP (Table 34).

However, analyzing correlations between pulmonary parameters and **disease duration** we observed a significant reduction of CVF, FEV1 and MEP (respectively $p= 0.0107$, $p =0.005$ and $p = 0.021$); the same correlation was not found for arterial blood gas parameters (Table 35).

In order to explore the interplay between neurological, respiratory and cardiac involvement, **we compared different groups of patients at T3, analyzing all the continuous variables registered**: age at onset, disease duration, BMI, grade of disability measured through Muscular Impairment Rating Scale (MIRS) and the Neuromuscular Impairment Function and Disability Scale (NFDS) score, the Epworth sleepiness scale, blood gas analysis values, respiratory function test, cardiological parameters (ejection fraction -EF-, PR and QRS interval length at ECG, Heart Rate) and sleep parameters (apnoea hypopnoea index -AHI-, oxygen desaturation index-ODI-, minimal and mean nocturnal saturation values–Min SaO2 and Mean SaO2) (table 37, 39, 41, 43). Comparing **patients with restrictive syndrome** (defined as CVF<80%) and **patients without restrictive syndrome** (CVF ≥80%) we did not find significant differences among the 3 classes of expansion (Table 36); we observed that patients with restrictive syndrome had higher BMI (p=0.0497), lower FEV1 (p< 0.0001), higher MIRS score (p=0.0004), higher NIFDS total score (p=0.0174) and motor domain and daily life score, longer PR interval length at ECG (p=0.0453), higher ODI index (p=0.0039), higher obstructive apnoea/hypopnoea and mixed apnoea index (p=0.0254), higher apnoea/hypopnoea index (AHI) (p= 0.0048) and lower nocturnal SaO2 values (p=0.0338, p=0.0095).

In the adjusted logistic regression, higher MIRS values were significant predictors for an increased risk of restrictive syndrome (OR=4.7, CI95% [1.56-14.13], p value 0.006), while PR and BMI were no longer associated with this outcome.

Moreover, exploring possible relationships between continuous variables using Spearman correlation coefficients we observed that:

- **CVF** showed positive correlations with age at onset (p=0.0179), FEV1 (p<0.0001), MEP (p=0.0455), pO2 (p=0,0066), nocturnal minimal and mean Sao2 (p=0.0024, p=0.0026) and with ejection fraction (p= 0.026); negative correlations were found with MIRS (p=0.0005), NIFDS total score (p=0.0004) and daily life score (p=0.0001), ODI index (p=0.0009) and obstructive AHI index and mixed apnoea index (p=0.0103).
- **FEV1** showed positive correlations with age at onset (p=0.0063), CVF (p<0.001), Min and Mean Sao2 (p =0.0101, p=0.0146) and negative correlations with MIRS (p=0.0008) and NIFDS total score (p = 0.0008).
- **MEP** showed positive correlations with age at onset (p=0,046) and CVF (p=0.0455); negative correlations with MIRS (p=0.0062), NIFDS total score (p<0.0001), Motor, Daily Life and Myotonia domains (p <0.0001, p=0.0014, p=0.0014) (Table B).

Comparing **patients with hypercapnia** (PaCO2 ≥45 mmHg) and **without hypercapnia** (PaCO2 <45 mmHg) we did not find significant differences among the 3 classes of expansion (Table 38); we found that patients with hypercapnia had higher score in myotonia subitem of the NIFDS scale (p=0.0082), lower SaO2 and pO2 (p =0.0355 and p=0.0233 respectively); no differences were found between the 2 groups of patients with respiratory function test or MIRS score (Table 39).

In the adjusted logistic regression we found that myotonia subitem of the NMDS was no longer associated with hypercapnia.

Applying Spearman's correlation we found that PaCO2 showed positive correlations with myotonia domain of the NIFDS scale (p=0.0276) and a negative correlations with pO2 and SaO2 (p=0.0008, p=0.0004) (Table B).

Comparing **patients with hypoxemia** (PaO2 < 80 mmHg) and **without hypoxemia** (PaO2 ≥ 80 mmHg) no differences were found among the 3 classes of expansion (table 40); we observed that patients with hypoxemia had higher BMI (p=0.0176) and higher pCO2 (p=0.0487), but we did not find significant differences with respiratory function test or MIRS score among the 2 groups of patients. However, using Spearman's correlation we observed that PaO2 showed positive correlations with CVF (p= 0.0066) and minimal and mean Sao2 (p=0.0464 and p=0.003), while negative correlations were found with BMI (p =0.0002), pCO2 (p=0.0008), NIFDS total score (p=0.0375), daily life (p=0.0164) and ODI (p=0.0146) (Table B).

In the adjusted logistic regression higher CVF values were significant predictors for a reduced risk of hypoxemia (OR= 0.96, CI 95%=[0.92-0.99], p-value= 0.035), while BMI was no longer associated.

Stratifying patients into 3 groups of MIRS score (**MIRS score I-II**, **MIRS score III**, **MIRS score IV-V**), we found a statistically significant difference among the 3 classes of expansion (E3 patients had higher score of MIRS) (p=0.0090) (Table 42). Patients with higher MIRS score had a lower age at onset (p 0.0328), a longer disease duration (p= 0.0005), higher score in NIFDS scale (p=0.0001) and daily life and motor domain, lower values of FEV1 (p=0.0076), CVF (p=0.0026) and MEP (p=0.0270), while PR interval length was longer (p=0.0028) (Table 43).

Table 25. Arterial blood gas (ABG) analysis and class of expansion– first evaluation (T1)

	E1 n (%)	E2 n (%)	E3 n (%)	Total	p
Normal	14 (60.87)	16 (47.06)	3 (60.0)	33 (53.23)	
Hypoxemia	2 (8.7)	3 (8.82)	0 (0.0)	5 (8.06)	
Hypercapnia	6 (26.09)	8 (23.53)	2 (40.0)	16 (25.81)	-
Hypoxemia + Hypercapnia	1 (4.35)	7 (20.59)	0 (0)	8 (12.9)	
Total	23 (100)	34 (100)	5 (100)	62 (100)	

Table 26. Arterial blood gas (ABG) analysis and class of expansion – last evaluation (T3)

	E1 n (%)	E2 n (%)	E3 n (%)	Total	p
Normal	12 (63.16)	14 (43.75)	4 (100)	30 (54.55)	
Hypoxemia	0 (0)	1 (3.13)	0 (0)	1 (1.82)	
Hypercapnia	3 (15.79)	10 (31.25)	0 (0)	13 (23.64)	-
Hypoxemia + Hypercapnia	4 (21.05)	7 (21.88)	0 (0)	11 (20)	
Total	19 (100)	32 (100)	4 (100)	55 (100)	

Table 27. Spirometry results and class of expansion – first evaluation (T1)

	E1 n (%)	E2 n (%)	E3 n (%)	Total	p
Normal	9 (37.5)	4 (10)	0 (0)	13 (18.31)	
Reduction of MIP or MEP	8 (33.33)	6 (15)	0 (0)	14 (19.72)	
Mild restrictive syndrome	4 (16.67)	14 (35)	2 (28.57)	20 (28.17)	**0.007**
Moderate restrictive syndrome	3 (12.5)	10 (25)	4 (57.14)	17 (23.94)	
Severe restrictive syndrome	0 (0)	5 (12.5)	1 (14.29)	6 (8.45)	
Total of patients	24 (100)	40 (100)	7 (100)	71 (100)	

Table 28. Spirometry results and class of expansion – last evaluation (T3)

	E1 n (%)	E2 n (%)	E3 n (%)	Total	p
Normal	7 (31.82)	6 (17.65)	0 (0)	13 (20.97)	-
Reduction of MIP or MEP	5 (22.73)	6 (17.65)	1 (16.67)	12 (19.35)	
Mild restrictive syndrome	5 (22.73)	9 (26.47)	1 (16.67)	15 (24.19)	
Moderate restrictive syndrome	5 (22.73)	7 (20.59)	3 (50)	15 (24.19)	
Severe restrictive syndrome	0 (0)	6 (17.65)	1 (16.67)	7 (11.29)	
Total of patients	22 (100)	34 (100)	6 (100)	62 (100)	

Table 29. Pulmonary parameters and class of expansion - first evaluation (T1)

	E1 median (IQR)	E2 median (IQR)	E3 median (IQR)	Total (IQR)	P
PCO2	43 (41-47)	45 (41-49)	43 (39-46)	43.5 (41-47)	-
PO2	86 (78-92)	87 (76-93)	93 (90-93)	88 (77-93)	-
SaO2	97 (96-97)	97 (95-98)	97 (96-98)	97 (96-98)	-
CVF	89 (82-94.5)	75 (61-89.4)	62 (56-74)	78 (68-92)	**0.0003**
FEV1	91 (80.5-97.5)	77.85 (61-90)	69.5 (55-73)	80.5 (69.5-93)	**0.0020**
MIP	41 (23-55.5)	27 (20-38)	16 (14-23)	27.5 (20-42)	**0.0178**
MEP	30 (19-41)	23 (17-31)	17 (17-18)	23 (17-32)	**0.035**

Table 30. Pulmonary parameters and class of expansion - last evaluation (T3)

	E1 median (IQR)	E2 median (IQR)	E3 median (IQR)	Total (IQR)	P
PCO2	44.05 (41-48)	46 (43-48.5)	43 (41-44.5)	45 (42-48)	-
PO2	84.1 (76-94)	85 (79-92)	81.5 (73.5-86.5)	85 (79-92)	-
SaO2	97.2 (94.5-98)	97 (96-97)	96.9 (95.4-97)	97 (96-97.5)	
CVF	89.5 (76.5-99)	76 (58-88)	60 (57-70)	78 (63-92)	**0.0085**
FEV1	84 (73.7-100)	70 (57-89)	61.5 (55-64)	78 (62-91)	**0.0040**
MIP	37 (26-48)	33 (28-58)	17.5 (6-29)	34 (26-48)	-
MEP	32 (25-40)	24 (20-33)	13 (5-21)	26 (21-35)	**0.0255**

Table 31. Pulmonary parameters and age at onset (T1)

	Congenital/Childhood onset	Juvenile/Adult onset	Late onset	Total	P
PCO2	47 (41-50)	44 (42-47)	42 (39-46)	44 (41-47)	-
PO2	90 (81-95)	86 (76.5-92)	80 (76-93)	87.5 (77-93)	-
SaO2	97 (96.5-98)	97 (96-97)	96 (95-98)	97 (96-98)	-
CVF	72 (62-75)	81 (68-92)	89.4 (77-94)	79 (68-92)	**0.0165**
FEV1	70.5 (58.5-85)	82 (68-93)	85 (78-94)	81 (69-93)	**-**
MIP	19 (13-32)	34 (22-50)	25 (20-42)	27 (20-42)	**0.050**
MEP	16 (7-23)	23.5 (17-34)	29 (21-43)	23 (17-32.5)	**0.0069**

Table 32. Pulmonary parameters and age at onset (T3)

	Congenital/Childhood onset	Juvenile/Adult onset	Late onset	Total	P
PCO2	46.5 (43-48.5)	45 (43-48)	42 (39-48)	45 (42-48)	-
PO2	86.5 (81-91)	83.5 (76-89)	94 (75-102)	85 (79-92)	-
SaO2	97 (96.4-97)	97 (95-97)	97.5 (95-98)	97 (96-97.5)	-
CVF	65 (57-81)	79 (63-91)	92 (76-103)	78 (63-92)	**0.014**
FEV1	61 (55-70)	79.5 (65.5-89.5)	82 (75-107.6)	78 (62-91)	**0.0069**
MIP	29 (19-30)	35.5 (28-50)	34 (25-53)	34 (26-48)	**-**
MEP	21 (15-22)	26 (22-37)	32 (25-53)	26 (21-35)	**0.0231**

Table 33. Pulmonary parameters at T1 and T3 and class of expansion

	First evaluation (T1)	**Last evaluation (T3)**	P
	Median (IQR)	**Median (IQR)**	
	E1 (N=24)		
CVF	89 (82-94.5)	89.5 (76.5-99)	-
FEV1	91 (80.5-97.5)	84 (73.7-100)	-
MIP	41 (23-55.5)	37 (26-48)	-
MEP	30 (19-41)	32 (25-40)	-
PCO2	43 (41-47)	44.1 (41-48)	-
PO2	86 (78-92)	84.1 (76-94)	-
SaO2	97 (96-97)	97.2 (94.5-98)	-
	E2 (N=41)		
CVF	75 (61-89.4)	76 (58-88)	-
FEV1	**77.9 (61-90)**	**70 (57-89)**	**0.0066**
MIP	**27 (20-38)**	**33 (28-58)**	**0.0054**
MEP	23 (17-31)	24 (20-33)	-
PCO2	45 (41-49)	46 (43-48.5)	-
PO2	87 (76-93)	85 (79-92)	-
SaO2	97 (95-98)	97 (96-97)	-
	E3 (N=7)		
CVF	62 (56-74)	60 (57-70)	-
FEV1	69.5 (55-73)	61.5 (55-64)	-
MIP	16 (14-23)	17.5 (6-29)	-
MEP	17 (17-18)	13 (5-21)	-
PCO2	43 (39-46)	43 (41-44.5)	-
PO2	93 (90-93)	81.5 (73.5-86.5)	-
SaO2	97 (96-98)	96.9 (95.4-97)	-

Table 34. Pulmonary parameters at T1 and T3 – total of patients

	First evaluation (T1)	**Last evaluation (T3)**	**p-value**
	Median (IQR)	**Median (IQR)**	
Epworth	8 (6-12)	7 (5-10)	
MIRS	**3 (3-4)**	**4 (3-4)**	**0.0232**
CVF	78 (68-92)	78 (63-92)	
FEV1	**80.5 (69.5-93)**	**78 (62-91)**	**0.0033**
MIP	**27.5 (20-42)**	**34 (26-48)**	**0.006**
MEP	**23 (17-32)**	**26 (21-35)**	**0.0419**
PCO2	43.5 (41-47)	45 (42-48)	
PO2	88 (77-93)	85 (79-92)	
SaO2	97 (96-98)	97 (96-97.5)	

Table 35. Pulmonary parameters and disease duration at T3

Pulmonary parameters	**P value**
CVF	**0.0107**
FEV1	**0.005**
MEP	**0.021**

Table 36. Distribution of patients/without restrictive syndrome and class of expansion

	E1 n (%)	E2 n (%)	E3 n (%)	Total	p
Without restrictive syndrome (CVF ≥80%)	12 (46.15)	13 (50)	1 (3.85)	26	-
With restrictive syndrome (CVF < 80%)	8 (24.24)	20 (60.61)	5 (15.15)	33	-
Total	20	33	6	59	-

Table 37. Comparison between patients with/without restrictive syndrome at last evaluation (T3)

	Without restrictive syndrome N=26	With restrictive syndrome N=33	p-value
Total of patients n (%)	**26 (100)**	**33 (100)**	
Females n (%)	13 (50)	14 (42.4)	-
	Median (IQR)	**Median (IQR)**	
Age at onset	26 (16-45)	25 (6-35)	-
Age at evaluation	49 (36.3-53.8)	50.6 (36.1-53.5)	-
Disease duration	15.1 (9.8-25.9)	20.7 (13.4-27.8)	-
MIRS	**3 (3-4)**	**4 (3-4)**	**0.0004**
NP_total	2.5 (1-5)	2.5 (1-6.5)	-
MO_total	**9 (6-13)**	**13 (9.5-15.5)**	**0.0483**
My_total	6 (4-7)	6 (5-8)	-
DL_total	**2 (0-3)**	**4 (2-6)**	**0.0032**
NIFDS total	**22 (12-26)**	**28.5 (19.5-32)**	**0.0174**
Epworth	9 (6-11)	7 (5-9)	-
BMI	**22.35 (20.8-25.3)**	**24.1 (23.4-25.9)**	**0.0497**
PCO	46 (42-50)	44 (41-47)	-
pO2	89 (80-94)	83 (76-89)	-
SaO2	97 (96-98)	97 (95-97)	-
CVF	**94 (88-101)**	**65 (57-74)**	**<0.0001**
FEV1_	**92 (86-107.6)**	**65 (55-73)**	**<0.0001**
MIP	34 (28.5-49)	33.5 (25-47)	-
MEP	28.5 (22.5-38)	24 (17-33)	-
HR mean media	66 (59-71)	69 (64-76)	-
HR min	42.5 (37-45)	43 (38.5-45.5)	-
HR max	121 (113-127)	127 (110.5-133)	-
PR interval length	**176 (156.5- 208.5)**	**203 (181-236)**	**0.0453**
QRS interval length	110 (105-122)	120.5 (102-144)	-
Ejection Fraction	61.8 (57.9-65)	60 (53-62.5)	-
Oxygen desaturation index	**4.5 (1.7-13.3)**	**16.7 (8.8-21,8)**	**0.0039**
Min SaO2 (nocturnal)	**86 (79-90)**	**80 (77-83)**	**0.0338**
Mean SaO2(nocturnal)	**94 (92.7-95.34)**	**92.8 (90.6-93.34)**	**0.0095**
Obstructive apnoea/hypopnoea index + Mixed apnoea	**4.9 (1.3-12.9)**	**14.9 (11.35-20)**	**0.0254**
Central apnoea index, n/h	0.4 (0-1.3)	1.02 (0.1-4.6)	-
Apnoea/Hypopnoea Index	**4.95 (2.99-13.25)**	**17.03 (10.17-22.6)**	**0.0048**

NIFDS = "Neuromuscular impairment function and disability scale" and its items: neuropsychological domain (NP), motor domain (MO), myotonia domain (MY) and daily life activity domain (DL).

Table 38. Distribution of patients with/without hypercapnia and class of expansion.

	E1 n (%)	E2 n (%)	E3 n (%)	Total	p
Without hypercapnia	10 (35.71)	14 (50)	4 (14.29)	28	-
With hypercapnia (pCO2>45)	8 (30.77)	18 (69.23)	0	26	-
Total	18	32	4	54	-

Table 39. Comparison between patients with/without hypercapnia at last evaluation (T3)

	Without hypercapnia	With hypercapnia	p-value
Total of patients n (%)	**28 (100)**	**26 (100)**	
Females n (%)	14 (50)	11 (42.3)	**-**
	Median (IQR)	**Median (IQR)**	
Age at onset	27 (19-41)	20.5 (6-34)	-
Age at evaluation	52.3 (45.7-56)	44.9 (33.7-55.4)	-
Disease duration	20.5 (11.5-31.4)	20.5 (11.9-29.4)	-
MIRS	4 (3-4)	3 (3-4)	-
NP_total	2 (1-5)	3 (1-6)	-
MO_total	8 (7-15)	13 (9-15)	-
My_total	**5 (3-6)**	**7 (5-8)**	**0.0082**
DL_total	2 (1-5)	3 (1-5)	-
NIFDS total	16 (13-30)	26 (22-32)	-
Epworth	7 (5-10)	8 (7-9)	-
BMI	23.7 (21.8-25.15)	25.25 (21-28.1)	-
pO2	**89 (79-95.9)**	**82.5 (76-88)**	**0.0355**
SaO2	**97 (96.2-98)**	**96 (95-97)**	**0.0233**
CVF	76 (61.5-97)	85 (63-91)	-
FEV1	73 (62-96)	81 (60-88)	-
MIP	30.5 (25-50)	35 (30-46)	-
MEP	28 (19-35)	25 (21-37)	-
HR mean	68.5 (59-75)	70 (62.5-73)	-
HR min	41 (38-45)	43 (34-45)	-
HR max	125 (115-140)	119 (112-127)	**-**
PR interval length	202 (163-224)	191.5 (173-219.5)	-
QRS interval length	125 (105.5-141.5)	110 (105-122)	-
Ejection Fraction	60.6 (56.5-63.1)	60.4 (56.3-65)	-
Oxygen desaturation index (ODI)	14.4 (5-20.3)	11.7 (4-21.8)	-
Min SaO2 (nocturnal)	81 (78-86)	81.5 (75-89)	-
Mean SaO2(nocturnal)	94 (91.5-95.2)	92.8 (89.97-94)	-
Obstructiveapnoea/hypopnoea + Mixed apnoea	12.6 (7.8-17.95)	8.1 (3.1-18.7)	-
Central apnoea index, n/h	0.4 (0-3.8)	0.8 (0-2.3)	-
Apnoea/Hypopnoea Index (AHI	13.3 (3.82-17.94)	6.39 (4.87-21.34)	-

Table 40. Distribution of patients with/without hypoxemia and classes of expansion at T3

	E1 n (%)	E2 n (%)	E3 n (%)	Total	p
Without hypoxemia	13 (35.14)	22 (59.46)	2 (5.41)	37	-
With hypoxemia	5 (31.25)	9 (56.25)	2 (12.5)	16	-
Total	18	31	4	53	-

Table 41. Comparison between patients with/without hypoxemia at last evaluation (T3)

	Without hypoxemia	With hypoxemia (pO2<80)	p-value
Total of patients n (%)	**37 (100)**	**16 (100)**	
Females n (%)	**18 (48.7)**	**7 (43.8)**	**-**
	Median (IQR)	**Median (IQR)**	
Age at onset	27 (12-37)	24 (16-41)	-
Age at evaluation_T3	50.6 (35.6-53.5)	52.6 (41.8-58.8)	-
Disease duration_T3	19.7 (11.9-27.8)	26.1 (13.8-30.3)	-
MIRS	3 (3-4)	4 (3.5-4)	0.0535
NP_total	2 (1-5)	4.5 (2-7)	-
MO_total	12.5 (7-15)	11.5 (9-16)	-
My_total	6 (4.5-8)	6 (5-7)	-
DL_total	2 (1-5)	4.5 (2-7)	-
NIFDS total	24 (13.5-30)	26 (20.5-38)	-
Epworth	7.5 (5-10)	7 (6-9)	-
BMI	**23.2 (21.3-25.3)**	**25.7 (24.9-28.2)**	**0.0176**
PCO2	**45 (40-47)**	**47.5 (43.5-51)**	**0.0487**
SaO2	**97 (97-98)**	**94 (93-95)**	**<0.0001**
CVF	80 (66.5-95.5)	62.5 (54-91)	-
FEV1	81 (67-95)	70.5 (56-82)	-
MIP	33 (25-46)	37 (34-59)	-
MEP	26 (21-39)	25 (15-33)	-
HR mean	69 (62-74)	69 (62-72)	-
HR min	43 (36-46)	41.5 (35.5-44.5)	-
HR max	122 (112-137)	120 (114-127)	-
PR interval length	188 (163-219)	215.5 (175-229.5)	-
QRS interval length	113 (106-134)	109.5 (96.5-135)	-
Ejection Fraction	60.5 (58-64.1)	61 (53-65)	-
Oxygen desaturation index (ODI)	11.2 (2-21)	19.7 (11.54-22.1)	-
Min SaO2 (nocturnal)	82 (77-89)	79 (67-81)	-
Mean SaO2(nocturnal)	94 (90.9-94.7)	93 (86-94)	-
Obstructive apnoea/hypopnoea index + Mixed apnoea	10.9 (3.4-17.2)	18.7 (4-36.2)	-
Central apnoea index, n/h	0.7 (0-3.8)	0.86 (0-2.3)	-
Apnoea/Hypopnoea Index (AHI)	11.46 (4.43-17.77)	19.3 (5.71-24.06)	-

Table 42. Patients with different MIRS score (I-II vs III vs IV-V) and class of expansion

	E1 n (%)	**E2 n (%)**	**E3 n (%)**	**Total**	p
MIRS 1-2	5 (83.33)	1 (16.67)	0	6	0.0090
MIRS 3	10 (37.04)	17 (62.96)	0	27	
MIRS 4-5	9 (23.08)	23 (58.97)	7 (17.95)	39	
Total	24	41	7	72	

Table 43. Comparison between patients with different MIRS score at last evaluation (T3)

	MIRS 1-2	**MIRS 3**	**MIRS 4-5**	
Total patients	6 (100)	27 (100)	39 (100)	
Females				
Variables	Median	Median	Median	P value
Age at onset	**43.5 (38-53)**	**20 (15-37)**	**25 (10-36)**	**0.0328**
Age at evaluation	**52.5 (52.3-53.8)**	**41.9 (29.3-52.2)**	**51.8 (42.8-56.7)**	**0.0466**
Disease duration	**9.8 (7.5-10.3)**	**14.9 (7.7-20.5)**	**27 (17-34.9)**	**0.0005**
NP_total	3 (0-5)	2 (1-3)	4 (1.5-6.5)	**-**
MO_total	**5 (3-5)**	**10 (7-13)**	**13.5 (10.5-16)**	**0.0005**
My_total	4 (3-5)	7 (5-8)	6 (5-8)	**-**
DL_total	**0 (0-0)**	**2 (1-3)**	**5 (3-6)**	**<0.0001**
NIFDS total	**10 (9-10)**	**20.5 (13-26)**	**30 (24.5-34)**	**0.0001**
Epworth	6 (6-10)	8.5 (6-10)	7 (5-9.5)	-
BMI	23.2 (22.8-23.7)	23.2 (20.8-26.3)	24.8 (21.9-26.2)	-
PCO2	43.1 (40-46)	47 (43-51)	45 (41-47)	-
pO2	85 (80.4-94)	89 (80-99)	85 (76-89)	-
SaO	96.8 (96-97.8)	97 (96-98)	97 (95-97)	-
CVF	**93 (85-112)**	**88 (72-99)**	**66 (58-79)**	**0.0026**
FEV1	**88 (82-114)**	**83 (70-93)**	**70 (55-81)**	**0.0076**
MIP	36 (21-36)	38 (25-53)	33 (28-42)	-
MEP	**39 (30-56)**	**30.5 (23-37)**	**23 (19-32)**	**0.0270**
HR mean	63 (60-74)	67 (59-76)	69.5 (62-75)	-
HR min	35 (33.5-39.5)	43 (37-45)	43.5 (40.5-46)	-
HR max	109 (100-112)	121 (115-132)	125 (108-134)	-
PR interval length	**146 (139-184.5)**	**177 (163-204)**	**210 (187-231)**	**0.0028**
QRS interval length	107 (96.5-115.5)	114 (99-133)	113 (105-166)	
Ejection Fraction	62.1 (61.5-63.8)	61 (56.8-66.7)	60 (52.7-63.5)	

Table B. Correlations between pulmonary parameters and other clinical and instrumental variables at T3

Parameter		Positive correlation		Negative correlation
CVF	Age at onset	0.0179	MIRS	0.0005
	FEV1	<0.0001	NIFDS scale total	0.0004
	MEP	0.0455	Motor domain and Daily Life domain (NIFDS)	0.003 0.0001
	pO2	0.0066	ODI	0.0009
	Ejection Fraction	0.026	Obstructive apnoea/hypopnoea index and mixed apnoea index	0.0103
	Nocturnal SaO2	0.0024		
FEV 1	Age at onset	0.0063	MIRS	0.0008
	CVF	<0.001	NIFDS scale total	0.0008
	Nocturnal saturation values (mean and min)	0.0101 0.0146		
MIP			NIFDS scale total	0.014
			Motor domain (NIFDS scale)	0.0074
MEP	Age at onset	0,046	MIRS	0.0062
	CVF	0.0455	NIFDS scale total	<0.0001
	MIP	0.0001	Motor domain (NIFDS scale)	<0.0001
			Daily Life (NIFDS scale)	0.0014
			Myotonia domain	0.0014
pCO2	Myotonia domain (NIFDS	0.0276	pO2	0.0008
			SaO2	0.0004
pO2	CVF	0.0066	BMI	0.0002
	SaO2	<.0001	pCO2	0.0008
	Nocturnal saturation min and	0.0464	NIFDS scale total	0.0375
		0.003	Daily Life(NIFDS scale)	0.0164
			ODI	0.0146
SaO2	Nocturnal SaO2 mean	0.0054	BMI	0.0004
			pCO 2	0.0004
			Daily Life(NIFDS scale)	0.0237

The table shows only significant correlations

4. SLEEP DISORDERS

EXCESSIVE DAYTIME SLEEPINESS (EDS)

Descriptive analysis

We administered the Epworth Sleepiness Scale (ESS) to 55 patients; pathological daytime sleepiness (defined as ESS score > 10) was present in 9/55 patients, respectively 4/17 (23.5%) E1, 5/34 (14.3%) E2 and 0/4 E3 patients (Table 45).

Correlation analysis

Comparing patients with and without excessive daytime sleepiness (EDS), we did not find significant differences among the 3 genetic classes (Table 44).

We found no significant associations between EDS and age at onset, disease duration, disability scales, arterial blood gas values, cardiological parameters, ODI or obstructive apnoea/hypopnoea/mixed apnoea index (Table 46).

We found a positive correlation among ESS score and Neuropsychological domain (NP) of the NIFDS scale (p =0.0116), COMPASS total score (p = 0.0002) and its subitems secretomotor, gastrointestinal and pupillomotor domain (p = 0.0476, p= 0.0146 and p = 0.001 respectively) and with autonomic innervation on skin biopsy (Table C). The positive association with higher CVF, FEV1 and nocturnal SaO2 was not confirmed after applying linear regression.

Table 44. Epworth sleepiness scale score and class of expansion

	E1 Median (IQR)	E2 Median (IQR)	E3 Median (IQR)	Total Median (IQR)	p
Epworth Sleepiness Scale	7.5 (6-12)	8.5 (6-12)	7 (4-8)	8 (6-12)	-

Table 45. Patients with/without excessive daytime sleepiness among the 3 classes of expansion

	E1 n (%)	E2 n (%)	E3 n (%)	Total
Absence of excessive daytime sleepiness (ESS < 10)	13 (28.26)	29 (63.04)	4 (8.7)	46
Excessive daytime sleepiness (ESS≥10)	4 (44.4)	5 (55.56)	0	9
Total	17	34	4	55

Table C. Correlations between Epworth Sleepiness Scale and other clinical and instrumental variables

Parameter		Positive correlation		Negative correlation
ESS total score	Neuropsychological domain (NP) NIFDS	0.0116		
	%autonomic fibres innervating the sweat	0.023		
	%autonomic fibres innervating the sweat	0.0253		
	COMPASS total score	0.0002		
	Secretomotor	0.0476		
	Gastrointestinal domain COMPASS	0.0146		
	Pupillomotor	0.001		

The table shows only significant correlations

Table 46. Comparison between patients with and without excessive daytime sleepiness at T3

	Without excessive daytime sleepiness (ESS<10)	**With excessive daytime sleepiness (ESS>10)**	**p-value**
Total of patients n (%)	**46 (100)**	**9 (100)**	
Females n (%)	20 (43.5%)	7 (77.8%)	**-**
	Median (IQR)	**Median (IQR)**	
Age at onset	26 (16-36)	16 (15-48)	-
Age at evaluation	50.7 (36.3-53.5)	47.3(35.5 - 53.8)	-
Disease duration	18.9 (12.6-27.8)	20 (4.2-23.1)	-
MIRS	4 (3-4)	3 (3-4)	-
NP_total	2 (1-5)	5 (3-6)	-
MO_total	11 (8-15)	13 (7-15)	-
My_total	6 (5-7)	70 (0-8)	-
DL_total	2 (1-5)	4 (0-5)	-
NIFDS total	24 (16-30)	28 (13-32)	-
Epworth	**7 (5-9)**	**13 (11-16)**	**<0.0001**
BMI	23.8 (21.45-25.8)	24.25 (21.75-26.35)	-
PCO2	45 (41-48)	44.5 (40-51)	-
pO2	86.1 (79-92)	90.5 (88-104)	-
SaO2	97 (96-97.5)	97 (97-98)	-
CVF	**77 (63-91)**	**93.5 (86.5-101)**	**0.0307**
FEV1	**73 (62-86)**	**93 (90-105)**	**0.0154**
MIP	34 (26-47)	29 (20-48.5)	-
MEP	24 (21-37)	30	-
HR mean media	69.5 (62-77)	60	-
HR min	42.5 (37-46)	41	-
HR max	125 (113-135)	116.5	-
PR interval length	202 (181-224)	175	-
QRS interval length	111 (99.5-133)	111	-
Ejection Fraction	60.2 (56.8-63.6)	61.2	-
Oxygen desaturation index (ODI)	11.2 (4-21.5)	5.3 (2.1-14.6)	-
Minimal SaO2 (nocturnal)	81.5 (77.5-89)	86 (82-89)	-
Mean SaO2(nocturnal)	**92.9 (90.7-94)**	**94.3 (93-96)**	**0.0492**
Obstructive apnoea/hypopnoea index + mixed apnoea index	11.5 (3.6-17.3)	8.4 (2.55-14.45)	-
Central apnoea index, n/h	0.7 (0-2.3)	0.45 (0-0.95)	-
Apnoea/Hypopnoea Index (AHI)	13.3 (4-19.3)	8.91 (4.34-14.6)	-

MIRS = muscular impairment rating scale

NIFDS= neuromuscular impairment function and disability scale (NIFDS); NP= neuropsychological domain, MO= motor domain, MY= myotonia domain, DL=daily life activity domain, HR heart rate

SLEEP RELATED BREATHING DISORDERS (SRBD)

Descriptive analysis

At the time of the first evaluation (T1) 53 patients underwent cardiorespiratory monitoring in order to assess the presence of sleep related breathing disorders (SRBD) and to differentiate between obstructive, mixed and central apnoea. Overall sleep apnoea was detected in 36/53 (67.9%) patients: 11/17 (64%) E1, 22/31 (70.96%) E2 and 3/5 (60%) patients with E3 expansion.

We observed a predominance of pure obstructive apnoea in 22/36 (61.1%) patients; pure central apnoea was detected in 2/36 (5.6%) patients and both obstructive and central apnoea were detected in 12/36 (33.34%) patients. In the E1 group most patients presented both central and obstructive events, while in the E2 and E3 group there was a predominance of pure obstructive apnoea.

In the E1 group of patients apnoea was mild ($5 \leq AHI \leq 15$) or severe ($AHI > 30$); in the E2 group the majority of patients had mild apnoea (35.48% of patients), while in the E3 group there was a predominance of severe apnoea (40% of patients) (Table 47).

At the time of the last evaluation (T3) 41 patients underwent the cardiorespiratory monitoring. Apnoea was detected in 10/14 (71.4%) E1, 17/22 (77.27%) E2 and 5/5 (100%) E3 patients. There was a predominance of obstructive apnoea in all genetic classes (Table 47).

Correlation analysis

Analyzing the type and severity of apnoea within the 3 genetic classes we did not find significant differences both at T1 and T3 (Table 47).

Analyzing the different sleep parameters oxygen desaturation index (ODI), minimal and mean nocturnal saturation (Min SaO2, Mean SaO2), obstructive apnoea/hypopnoea and mixed apnoea index, central apnoea index at T1 and T3, we found no differences between the 3 genetic classes, except for a mild reduction of mean nocturnal saturation at T3 in the E2 class of patients (Table 48).

Comparing **patients with and without sleep apnoea** (according to polysomnographic scores) at T3, we observed that patients with sleep apnoea had a higher BMI (p=0.0083) and lower CVF values (p=0.0402) than patients without sleep apnoea; no differences were detected among the 2 groups regarding age at onset, disease duration, disability scales, arterial blood gas values or cardiological parameters (Table 50).

In the adjusted logistic regression, higher CVF values were significant predictors for a reduced risk of apnoea, (OR=0.93, CI 95%= [0.87-0.99], p-value =0.023).

Moreover, applying Spearman's correlation we observed that (Table D):

- **ODI** showed significant positive association with BMI (p=0.0004), MIRS (p=0.0096), NIFDS total score (p = 0.0273) and daily life score (p=0.0273), PR and QRS interval length at ECG (p=0.0147, p=0.022), obstructive AHI and mixed apnoea index (p<0.0001); negative correlations were found with diurnal pulmonary parameters CVF, FEV1, pO2 and SaO2 (p=0.0009, p=0.0032, p=0.0146, p=0.0351) and with nocturnal Min SaO2 and Mean SaO2 (p=0.0115, p<0.0001).

In the adjusted linear regression for age, sex and disease duration we found that ODI increased with age when corrected for the other variables; the positive association with daily life subitem of NIFDS (β=+2.9, p-value=0.001) and the negative associations with CVF (β= -0.35, p-value=0.00), FEV1 (β= -0.27, p-value=0.002) and pO2 (β= -0.50, p-value=0.006) were confirmed, while the associations with MIRS, PR and QRS were no longer significant.

- Obstructive AHI and mixed apnoea index showed significant positive correlations with BMI (p=0.0021), MIRS (p=0.014), Daily Life score of the NIFDS scale (p=0.0283), PR and QRS interval length on ECG (p= 0.0037); negative correlations were found with diurnal pulmonary parameters CVF and FEV1 (p=0.0103, p= 0.0351) and with nocturnal Min SaO2 and Mean SaO2 (p<0.0001).

In the adjusted linear regression, the positive association with DL (β= 1.67, p-value=0.05) and PR (β= 0.09, p-value=0.05) and the negative association with CVF (β= -0.28, p-value=0.003) and FEV1 (β= -0.17, p-value=0.045) were confirmed; on the contrary the associations with MIRS and QRS were no longer significant.

-Nocturnal Minimal SaO2 and Mean SaO2 showed significant positive correlations with diurnal pulmonary parameters CVF, FEV1 and pO2; in addition, Mean SaO2 showed positive correlation with Heart Rate max (p=0.0184) and diurnal SaO2 (p= 0,0054); both Min SaO2 and Mean SaO2 showed negative correlation with BMI; in addition, Min SaO2 showed negative correlation with MIRS (p =0.0487) and with daily life score of the NIFDS scale (p= 0.0042).

In the adjusted linear regression, the positive association of minimal SaO2 with CVF (β=0.17, p-value=0.01), FEV1 (β=0.12, p-value=0.04) and diurnal pO2 (β=0.25, p-value=0.047) were confirmed; on the contrary the association with MIRS was no longer significant. The positive associations between mean SaO2 and CVF (β= 0.08, p-value= 0.003), FEV1 (β=0.06, p-value= 0.03), diurnal pO2 (β=0.16, p-value= 0.004), SaO2 (β=1.16, p-value= 0.007) and HR (β=0.04, p-value=0.015) were also confirmed.

Table 47. Type and severity of apnoea and expansion size at T1 and T3

T1 Total patients 53 (100)					T3 total patients 41 (100)			
Type of apnoea	**E1**	**E2**	**E3**	**p-value**	**E1**	**E2**	**E3**	**p-value**
Central apnoea	1 (5.88)	1 (3.23)	0	-				-
Central and obstructive events	6 (35.29)	5 (16.13)	1 (20)		2 (14.29)	3 (13.64)	2 (40)	
Obstructive apnoea	4 (23.53)	16 (51.61)	2 (40)		8 (57.14)	14 (63.64)	3 (60)	
Absence of apnoea	6 (35.29)	9 (29.03)	2 (40)		4 (28.57)	5 (22.73)	0	
Severity of apnoea	**E1**	**E2**	**E3**	**p-value**	**E1**	**E2**	**E3**	**p-value**
Mild	4 (23.53)	11 (35.48)	0	-	7 (50)	6 (33.33)	1 (25)	-
Moderate	3 (17.65)	9 (29.03)	1 (20)		2 (14.29)	5 (27.78)	2 (50)	
Severe	4 (23.53)	2 (6.45)	2 (40)		1 (7.14)	3 (16.67)	0	
Absence of apnoea	6 (35.29)	9 (29.03)	2 (40)		4 (28.57)	4 (22.22)	1 (25)	
Total	**17**	**31**	**5**		14	18	4	

Table 48. Sleep parameters at T1 and T3 in the different classes of expansion

	T1 Median (IQR)	**T3 Median (IQR)**	**P-VALUE T1-T3**
E1 (N=24)			
Oxygen desaturation index (ODI)	7.1 (3.1-30)	8.1 (3.9-14.4)	-
Min SaO2 (nocturnal)	83.5 (78-90.5)	86 (81-89)	-
Mean SaO2(nocturnal)	93.39 (92-95.4)	92.9 (91.5-94)	-
Obstructive apnoea/hypopnoea + Mixed apnoea	3.9 (1.15-14)	10.6 (3.8-12.6)	-
Central apnoea index, n/h	1 (0.4-11)	0.6 (0-1.55)	-
Apnoea/Hypopnoea Index (OHI)	4.5 (2-14.5)	8.34 (4.9-14.4)	-
E2 (n=41)			
Oxygen desaturation index (ODI)	8.8 (2.05-16.35)	11.7 (2.1-21.8)	-
Min SaO2 (nocturnal)	82 (74-88)	80 (75-89)	-
Mean SaO2(nocturnal)	94 (90.7-95.2)	94 (90.2-95.2)	**0.0160**
Obstructive apnoea/hypopnoea + Mixed apnoea	3.9 (1.7-11.8)	11 (3.1-18.7)	-
Central apnoea index, n/h	1.15 (0.5-3.6)	0.7 (0-3.8)	-
Apnoea/Hypopnoea Index (OHI)	5.72 (2.2-16.2)	14.77 (3.08-22.6)	-
E3 (N=7)			
Oxygen desaturation index (ODI)	20.7 (6-21.5)	16.6 (14.4-20.3)	-
Min SaO2 (nocturnal)	80 (73-82)	79.5 (76.5-82.5)	-
Mean SaO2(nocturnal)	92.45 (90.5-93.95)	93 (90.9-93)	-
Obstructive apnoea/hypopnoea + mixed apnoea index	13.5 (2.3-18.1)	17.2 (13-39)	-
Central apnoea index, n/h	4.7 (1.1-10)	0.4 (0.4-17.2)	-
Apnoea/Hypopnoea Index (OHI)	22.8 (3.4-23.7)	15.45 (13.3-17.6)	-

Table 49. Sleep parameters at T1 and T3 – total of patients

	p-value E1 vs E2 vs E2 T1	**p-value E1 vs E2 vs E2 T3**
Oxygen desaturation index (ODI)	0.5032	-
Min SaO2 (nocturnal)	0.5550	-
Mean SaO2(nocturnal)	0.5715	-
Obstructive apnoea/hypopnoea + mixed apnoea index	0.7740	-
Central apnoea index, n/h	0.5309	

Table 50. Comparison between patients with/without apnoea at last evaluation (T3)

	Without apnoea	**With apnoea**	
Total of patients n (%)	**9 (100)**	**32 (100)**	
Females n (%)	4 (44)	17 (53.1)	**-**
	Median (IQR)	**Median (IQR)**	**p-value**
Age at onset	16 (12-30)	24.5 (6-36.5)	-
Age at evaluation	35.56 (26.77-47.05)	47.11 (35.87-55.35)	-
Disease duration	14.51 (10.34-17.05)	20.45 (11.92-30.83)	-
MIRS	3 (3-4)	4 (3-4)	-
NP_total	3 (1.5-4.5)	4 (1-7)	-
MO_total	12.5 (9.5-13)	12 (7-15)	-
My_total	7 (6.5-7.5)	6 (5-8)	-
DL_total	2.5 (1.5-3)	5 (1-5)	-
NIFDS total	25 (21.5-27)	28 (15-35)	-
Epworth	8 (7-10)	9 (6-10.5)	**-**
BMI	**21.6 (21.06-23)**	**25.3 (21.9-27.3)**	**0.0083**
PCO2	46 (39-49)	46 (42.5-48)	-
pO2	90 (85-94)	85 (79.5-89)	-
SaO2	97 (96.4-98)	97 (96-97)	-
CVF	**91 (81-101)**	**75.5 (59-88)**	**0.0402**
FEV1	84 (81-109)	72.5 (61-89)	-
MIP	30 (25.5-36)	36 (28-53)	-
MEP	24 (22-28)	30 (21-39)	-
HR mean	75.5 (58.5-82)	68 (60.5-73.5)	-
HR min	43 (36-47)	41 (37-44)	-
HR max	132.5 (124-145.5)	119 (102-129)	-
PR interval length	177 (142-220)	196 (171-236)	-
QRS interval length	109 (88-116)	116 (106-133)	-
Ejection Fraction	60 (55-61.2)	60.85 (57-65)	-
Oxygen desaturation index (ODI)	1.7 (0.6-2.1)	14.5 (7.7-21.65)	**<0.0001**
Min SaO2 (nocturnal)	90 (89-90)	81 (75-85)	**0.0002**
Mean SaO2(nocturnal)	94.75 (94-96)	92.75 (90.7-94)	**0.0017**
Obstructive apnoea/hypopnoea index + Mixed apnoea	0.85 (0.2-3.45)	12.6 (10-18.7)	**<0.0001**
Central apnoea index, n/h	0 (0-0.5)	0.91 (0.2-3.85)	**0.0216**

Table D. Correlations between sleep parameters and other clinical and instrumental variables at T3

Parameter		Positive correlation		Negative correlation
Oxygen desaturation index (ODI)	MIRS	0.0096	CVF	0.0009
	PR	0.0147	FEV1	0.0032
	QRS	0.022	pO2	0.0146
	BMI	0.0004	SaO2	0.0351
	Daily Life domain (NIFDS)	0.0273	Min SaO2 (nocturnal)	0.0115
	NIFDS total	0.0273	Mean SaO2 (nocturnal)	<0.0001
	Obstructive apnoea/hypopnoea/ mixed apnoea index	<0.0001		
Min SaO2 (nocturnal)	CVF	0.0024	MIRS	0.0487
	FEV1	0.0101	BMI	0.0002
	pO2	0.0464	Daily Life domain (NIFDS)	0.0042
	Mean SaO2 (nocturnal)	<0.0001	Obstructive apnoea/hypopnoea/mixed apnoea index	<0.0001
Mean SaO2 (nocturnal)	HR max	0.0184	BMI	0.003
	CVF	0.0026	ODI	<0.0001
	FEV1	0.0146		
	pO2	0.003		
	SaO2	0.0054		
	Min SaO2 (nocturnal)	<0.0001		
	Obstructive apnoea/hypopnoea/mixed apnoea index	<0.0001		
Obstructive apnoea/hypopnoea/mixed apnoea index	MIRS	0.014	CVF	0.0103
	PR	0.0037	FEV 1	0.0351
	QRS	0.0037	Min SaO2 (nocturnal)	<0.0001
	BMI	0.0021	Mean SaO2 (nocturnal)	<0.0001
	Daily Life domain (NIFDS)	0.0283		

The table shows only significant correlations

5. SKIN BIOPSY

Descriptive analysis

To assess the grade and type of peripheral nervous system involvement 52 patients underwent electromyography; 23/52 (44.23%) patients had only myotonic discharges, 22/52 (42.3%) patients showed myotonic discharges and a myopathic pattern, while in 7/52 (13.46%) patients we found a polyneuropathy variably associated with myotonic discharges and/or myopathic pattern. In order to analyze somatic and autonomic unmyelinated skin fibres and to assess the possible presence of a small fibre polyneuropathy (SFP) 45 patients underwent a skin biopsy; we excluded from this part of the study patients with diabetes mellitus (a possible metabolic cause of SFP). The same patients were interviewed with the Composite Autonomic Symptom Score (COMPASS 31 score) and the questionnaire Douleur Neuropathique en 4 questions (DN4).

We first analyzed intraepidermal somatic nerve fibre density; then we analyzed the percentage of unmyelinated autonomic fibres innervating the sweat glands (sudomotor innervation) and arrector pili muscles (pilomotor innervation), which are found in the deep dermal and subcutaneous layers of skin.

A subclinical small fibre neuropathy (SFN) was observed in 29/45 patients (82%): in 5 patients we found a mixed autonomic and somatic SFN, in 24 patients there was a prevalent somatic involvement (Table 52).

We compared patients to a group of 19 healthy control subjects, matched for age and sex. We found that patients had a significantly lower density of somatic fibres compared to controls, while we did not find significant differences regarding autonomic fibres (Table 51); for this reason, for correlation analysis we analyzed only somatic innervation.

Correlation analysis

Analyzing epidermal nerve fibre density, no significant differences were found among the 3 classes of expansion (E1, E2, E3) nor among different groups of age at onset (Tables 53 and 54) or different groups of MIRS scale (Table 57). We observed that males presented a significant lower fibre density in the leg compared to females (p= 0.0288) (Table 55) and that fibre density was lower in the leg compared to the thigh (Table 56). The adjusted linear regression confirmed the negative association between male sex and lower leg innervation values (β=-2.67, CI 95%=[-5.16 / -0.18], p-value=0.036).

We studied the possible correlations among epidermal nerve fibre density and the different neurological, pneumological, cardiological and sleep parameters (age at onset, age at evaluation, disease duration, disability scales MIRS and NIFDS scale, Epworth, BMI, pCO2, pO2, SaO2, CVF, FEV1, MIP, MEP, HR mean, HR min, HR max, PR interval length, QRS interval length, ejection fraction, oxygen desaturation index, Nocturnal saturation, obstructive apnoea/hypopnoea index + mixed apnoea index, central apnoea index). No significant associations were found regarding these parameters, nor with the COMPASS 31 score.

The possible correlations among COMPASS 31 score and the other clinical and instrumental parameters are shown in Table 61. No patient reported pain with neuropathic features.

We also analyzed, using primary antibodies (anti-Pan Sodium Channel), the expression of sodium ion channel (Nav8) in skin biopsies of a small sample of DM1 patients and controls, but without significant differences between the 2 groups.

Table 51. Case control study

	Healthy controls n= 19	Patients n= 46	**P-value**
Variable	Median (IQR)	Median (IQR)	
Intraepidermal somatic nerve fibre density_leg	13.1 (11.1-15.1)	8.6 (5.7-11.6)	**0.0003**
Intraepidermal somatic nerve fibre density_thigh	20.5 (15.8-21.8)	13.9 (9.8-16)	**<0.0001**
%autonomic fibres innervating the sweat glands_leg	13.61 (12.67-14.2)	13.05 (11.97-14.61)	0.4353
%autonomic fibres innervating the sweat glands_thigh	14.91 (13.45-16.67)	15.26 (13.79-16.35)	0.6304
%autonomic fibres innervating arrector pili muscles_leg	15.3 (12.51-18.27)	15.65 (12.42-18.85)	0.8806
%autonomic fibres innervating arrector pili muscles_thigh	17.32 (15.15-20.56)	19.78 (18.07-21.84)	0.0871

Table 52. Skin biopsy and class of expansion

Skin Biopsy	**E1**	**E2**	**E3**	Total	P
Normal	5 (35.7%)	9 (33.3%)	2 (50%)	16 (35.6%)	0.613
Somatic fibre involvement	7 (50%)	16 (59.3%)	1 (25%)	24 (53.3%)	
Somatic and autonomic fibre involvement	2 (14.3%)	2 (7.4%)	1 (25%)	5 (11.1%)	
Total of patient tested	14 (100%)	27 (100%)	4 (100%)	45 (100%)	

Table 53. Intraepidermal somatic nerve fibre density and class of expansion

	E1 (N=24)	**E2 (N=41)**	**E3 (N=7)**	p-value
	Median (IQR)	Median (IQR)	Median (IQR)	
Intraepidermal somatic nerve fibre density_thigh	13.2 (10.25-14.45)	13.7 (7.9-16.3)	16.7 (9.8-17)	0.6607
Intraepidermal somatic nerve fibre density_leg	9.6 (6.8-11.3)	8.3 (5.7-12)	10.9 (5-13.8)	0.7683

Table 54. Intraepidermal somatic nerve fibre density and age at onset

	Congenital /Childhood onset (N=14)	**Late onset (N=18)**	**Juvenile/Adult onset (N=40)**	p-value
	Median (IQR)	Median (IQR)	Median (IQR)	
Intraepidermal somatic nerve fibre density_thigh	13.9 (11.15-16.4)	13.9 (7.7-14.5)	12.3 (9.5-15.4)	0.8383
Intraepidermal somatic nerve fibre density_leg	8.6 (8.1-12.35)	9.95 (5.7-13.5)	7.65 (5.3-11.3)	0.3674

Table 55. Intraepidermal somatic nerve fibre density and sex

	F (N=35)	**M (N=37)**	p-value
	Median (IQR)	Median	
Intraepidermal somatic nerve fibre density_thigh	14.1 (10-16.3)	12.4 (7.9-14.5)	0.3463
Intraepidermal somatic nerve fibre density_leg	**9.6 (7.9-13.5)**	**7.1 (5-10.9)**	**0.0288**

Table 56. Intraepidermal somatic nerve fibre density: comparison thigh and leg

fibre density_thigh	**fibre density_leg**	
Median	Median	p-value
13.9 (9.8-16)	**8.6 (5.7-11.6)**	**<0.0001**

Table 57. Intraepidermal somatic nerve fibre density and disability (MIRS scale)

	MIRS <= 3 (N=33)	**MIRS >3 (N=39)**	p-value
	Median (IQR)	Median (IQR)	
Intraepidermal somatic nerve fibre density_thigh	13.9 (10.1-14.5)	13.7 (8.85-16.8)	0.8143
Intraepidermal somatic nerve fibre density_leg	8.75 (6.8-11.4)	8.35 (5.35-12.5)	0.8109

6. PUPILLOMETRY

Descriptive analysis

We analyzed pupillometry data of 26 DM1 patients. Both eyes were evaluated separately.

All patients underwent a standard ophthalmological examination including optical coherence tomography that excluded ocular conditions which may interfere with the pupillometric evaluation.

Patients were compared to a group of 21 healthy control subjects, matched for age and sex; patients had significantly lower pupil size at baseline and a lower constriction response to light (peak amplitude) (Table 59).

Correlation analysis

Pupil size at baseline showed significant positive correlations with age at onset (p = 0.0351) and the cardiac parameter ejection fraction (p = 0.0215) (lower pupil size was associated with lower age at onset and lower ejection fraction value). Furthermore, a negative correlation was found with disease duration (p =0.0116), PR and QRS interval length (p = 0.0469 and p = 0.0067 respectively) (thus, lower pupil size was associated with longer disease duration and longer PR and QRS interval length at ECG). After applying the adjusted linear regression the positive association between pupil size at baseline and ejection fraction (β=0.09, CI 95%= [0.02 /0.15], p-value=0.011) and the negative association with QRS (β= -0.029, CI 95%=[-0.05/-0030], p-value=0.027) were confirmed.

No significant correlations were found with the other clinical parameters, skin biopsy parameters, nor Composite Autonomic Symptom Score (COMPASS 31) (Table 60).

Peak amplitude values showed significant positive correlations with the percentage of autonomic fibres innervating arrector pili muscles in the thigh (p = 0.0338) (higher constriction response values were associated with higher percentage of autonomic fibres), whereas a negative correlation was found with MIRS score (p = 0.0024), neuropsychological domain of the NIFDS scale (p = 0.0092) and with obstructive apnoea/hypopnoea index and mixed apnoea index (p =0.0232) (lower constriction response values were found in patients with higher disability score and higher apnoea/hypopnoea index) (Table 60).

In the adjusted linear regression for age, sex and disease duration the negative association with MIRS was confirmed (β= -0.76, CI 95%=[-0.12/-0.03], p-value=0.002), while the positive association with the percentage of autonomic fibres (β=0.02, CI 95%=[-0.00/-0.04], p-value=0.091) and the negative association with NP domain (β = -0.018, CI 95%=[-0.39/-0.01], p-value=0.064) were no longer significant; however, given the very low p-values, the loss of this association could be explained by the small sample of patients.

Table 59. Case control study

	Healthy controls	**Patients**	
Variable	Median	Median	p-value
Mean_baseline	6.52 (5.9-6.73)	5.18 (4.68-5.74)	0.0007
Peak_Amplitude_mean	0.5 (0.48-0.52)	0.44 (0.41-0.47)	<0.0001

Table 60. Pupillometry and correlations with clinical and instrumental parameters

Parameter		**Positive correlation**		**Negative correlation**
Mean_baseline	Age at onset	**0.0351**	Disease duration	**0.0116**
	Ejection Fraction	**0.0215**	PR length	**0.0469**
			QRS length	**0.0067**
Peak_Amplitude_mean	**% autonomic fibres**	**0.0338**	MIRS	**0.0024**
			NP domain of the NIFDS	**0.0092**
			Obstructive apnoea/hypopnoea index and mixed apnoea index	**0.0232**

The table shows only significant correlations

Table 61. Correlation analysis - clinical scales (COMPASS31, MIRS AND NIFDS)

COMPASS 31 score (Composite Autonomic Symptom Score)

Parameter		**Positive correlation**		**Negative correlation**
COMPASS total	Epworth	0.0002	PR interval length ECG	0.0332
	NP domain of the NIFDS	0.0152		
Orthostatic intolerance	HR mean	0.0031		
	Motor domain NIFDS	0.0066		
	Daily Life domain NIFDS	0.0108		
	NIFDS total score	0.0114		
Vasomotor	-	-	-	-
Secretomotor	Epworth	0.0476		
	CVF	0.0151		
	FEV1	0.045		
	%autonomic fibres innervating arrector pili muscles_leg	0.0471		
Gastrointestinal—mixed upper and diarrhoea and constipation	Epworth	0.0146	PR interval length ECG	0.0157
	Motor domain NIFDS	0.0317	ODI index	0.0115
	Myotonia domain NIFDS	0.0093	Apnoea/hypopnoea index	0.0014
Bladder	-	-	-	**-**
Pupillomotor	Epworth	0.001		
	%autonomic fibres innervating the	0.0044		

Table 62. Neuromuscular Impairment Function and Disability Scale (NIFDS)

Parameter		**Positive correlation coefficient (p value)**		**Negative correlation coefficient (p value)**
Neuropsychological domain (NP)	Ejection Fraction	0.4756	Peak amplitude (Pupillometry)	0.0092
	Epworth	0.0116		
	COMPASS total score	0.0152		
Motor domain (MO)	MIRS	0.0001	Age at onset	<.0001
	Orthostatic intolerance COMPASS	0.0066	Ejection Fraction	0.049
	Gastrointestinal domain COMPASS	0.0317	CVF	0.003
			FEV1	0.0028
			MIP	0.0074
			MEP	<0.0001
Myotonia domain (MY)	pCO2	0.0276	Age at onset	<.0001
	Gastrointestinal domain COMPASS	0.0093	Ejection Fraction	0.0307
			MEP	0.0014
Daily life activity domain (DL)	MIRS	<.0001	Age at onset	0.0006
	PR interval length ECG	0.0136	CVF	0.0001
	BMI	0.0196	FEV1	0.0012
	ODI index	0.0028	MIP	0.0351
	Apnoea/hypopnoea index	0.0283	MEP	<0.0001
	Orthostatic intolerance domain COMPASS	0.0108	pO2	0.0164
			SaO2	0.0237
			Sa O2 min nocturnal	0.0042
NIFDS total score	MIRS	<.0001	Age at onset	<.0001
	Orthostatic intolerance domain COMPASS	0.0114	CVF	0.0004
	ODI index	0.0273	FEV1	0.0008
			MIP	0.014
			MEP	<.0001
			pO2	0.0375
			SaO2	0.0607

Table 63. Muscular Impairment Rating Scale (MIRS)

Parameter		**Positive correlation coefficient (p-value)**		**Negative correlation coefficient (p-value)**
MIRS total score	PR interval length ECG	0.54075	CVF	0.0005
	Motor domain NIFDS	0.0001	FEV1	0.0008
	Daily Life domain NIFDS	<0.0001	MEP1	0.0062
	NIFDS total score	<0.0001	Peak amplitude (Pupillometry)	0.0024
	ODI index	0.0096	Min nocturnal SaO2	0.0487
	Apnoea/hypopnoea index	0.014		

7. NEUROPSYCHOLOGICAL AND PSYCHOLOGICAL ASSESSMENT

Clinical and genetic features

Among 37 patients with DM1 diagnosis, 14 belonged to E1, 21 belonged to E2 and two to E3 classes.

After quality control of images by visual assessment, three DM1 patients (one for each genetic class) were excluded because of suboptimal image quality due to motion artefacts.

Only E1 and E2 DM1 patients were included in the analyses. The final cohort of DM1 patients included 33 subjects (age, mean±standard deviation, 46.6±12.1 years, 19/14 F/M), divided into the two subgroups E1 (N=13, age 50.2±10.4 years, 7/6 F/M) and E2 (N=20, age 44.2±12.79 years, 12/8 F/M).

A group of 33 matched healthy controls was also included (age 45.8-12.83 years, 19/14 F/M). Healthy controls were selected from the database of the Neuroimaging Laboratory, designed to collect normative values of quantitative MR parameters for clinical and research purposes. Education level of cases was 13.86 ±3.24 years.

All patients performed a full neuropsychological evaluation and the psychological evaluation was completed for 33 out of 37 patients: for two patients, caregivers did not return the questionnaires and for two patients, the psychological scales pertaining to the mood domain were missing.

Comparing performance of overall DM1 patients (E1 and E2 classes) to the normal values, only 3/35 (8.57%) patients score lower than cut-offs in MMSE, while five patients (14.29%) were below cut-offs in CPM-47 test.

Performance in verbal short-term memory was below cut-offs in Digit Span forward (25.71%), B RST_immediate recall (11.43%) and RAVL_immediate recall (8.57%), while performance in visuospatial short-term memory (Corsi) was below norms for 31.43% of DM1 patients.

Long-term verbal memory was below cut-offs for 22.86% (BSRT_delayed recall) and 8.57% (RAVL_delayed recall).

Among the language tasks, a higher ratio of patients scores worse in associative fluency task (22.86%) compared to their performance in both categories word fluency task (8.57%) and BNT-short version (8.57%).

Performance in tasks involving executive functions was below cut-off for FAB (48.57%), Stroop test (34.29%) and Digit span backward (28.75%). Attention scores were worse than norms for a high percentage of DM1 patients: 25.71% in Attentional Matrices test and 40% in SDMT.

More than half of the sample (51.43%) were significantly impaired in a visuoconstructional task (ROCF_copy), while a significant ratio showed worsened performance in SCT (17.14%) and BJLOT (28.57%) visuoperceptual tasks. A discrete percentage of patients performed worse than controls (8.57%) in the Eyes Mind test investigating social cognition. A proportion of 11.43% of DM1 patients scored above cut-offs for the presence of anosognosia (MAI).

Regarding mood parameters, 28.57% of DM1 patients presented signs of depression (BDI-II), 40% presented above cut-off levels for the presence of trait anxiety and 45.71% showed high levels of state anxiety. A total of 20% presented higher levels of state anger, while for 42.86% higher anger expression was present as a personality trait.

Almost half (48.57%) of the DM1 sample presented significant levels of apathy.

Cognitive reserve index showed a mean total score of 102 (±11.77) belonging to the mean level of CRI in an age- and sex-matched control population.

A proportion of 91.43% of DM1 reported worsened quality of life (SF-36) during the last twelve months (HC index), 45.71% reported a significant level of physical pain and 45.71% reported worsened ratio of energy/fatigue in daily activities.

As concerns the comparison between E1 and E2 performance on each neuropsychological and psychological test, results showed worse performance of E1 patients in the visuoperceptual ability (Street test, E1 =7.64±2.21; E2 = 9.95±3.20; p <.05).

Performance in social cognition tasks was worse in E1 patients, both when asked to attribute an emotional label to pictures of eyes (Eyes test, E1 =22.86±2.25; E2 = 19.86±4.52; p <.05), and to attribute emotion and intentions to the characters of short stories (SET task E1 =15.57±1.55; E2 = 16.65±1.50; p <.05).

The assessment of state and trait anxiety (STAI-Y) showed a significant index of mild trait anxiety in E2 patients only (E1 =34.08±8.76; E2 = 42.05±10.03; p <.05), suggesting a higher predisposition to react anxiously to a situation.

Global score of apathy was significantly higher in E2 patients (15.68±4.60) than in E1 patients (11.77±4.13; p < 0.01). Thirteen of 19 E2 patients (68.42 %) met the criterion for apathy, contrasting with only four of the 13 (30.77 %) E1 patients.

All cognitive reserve indices were significantly higher in E1 patients compared to E2 patients (all Ps <.05), showing the presence of a higher number of protective factors, such as educational level, occupation and leisure time activities, enhancing the ability to optimize and maximize cognitive performance.

As concerns the perception of the impact of the disease on their quality of life, E1 patients reported a generally higher level of quality in all investigated domains, and significantly than E2 patients as concerned the domains of physical health (E1 =88.46±19.41; E2 =65.00±36.63), perceived limits due to emotional problems (E1 =87.18±21.68; E2 =62.77±17.55) and emotional well-being (E1 =75.38±13.74; E2 =65.00±36.63).

The results of the neuropsychological and psychological test and the comparison between the performance of E1 and E2 subgroups of patients on each test are reported on Table 64.

8. BRAIN MRI ACQUISITION AND ANALYSIS PROTOCOL

Significant results for brain regions volumes group comparisons are reported in Table 65 and shown in Figure 1. In addition to an enlargement of the lateral ventricular volumes, we detected at the subcortical level a bilateral decrease volume of accumbens and putamen nuclei in DM1 patients compared to healthy subjects, and a decrease of volumes in frontal, parietal, temporal and occipital cortices. Some regions also showed differences between the two subgroups of DM1 patients, such as the isthmus cingulate and the transverse temporal cortices and the superior parietal and temporal gyri (trend level), with volume reduction being more severe in E2 patients.

White matter lesion load was 3263±2377 mm^3 (mean±SD) in E1 patients (926-8565 mm^3, range), and 5164±9897 mm^3 in E2 patients (615-46394 mm^3,range), but without a significant effect of group after multiple comparisons correction.

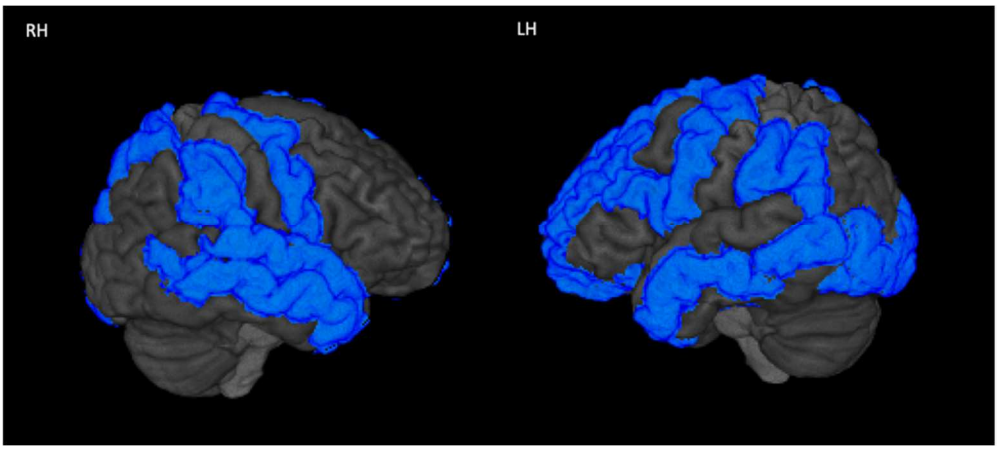

Figure 1. Cortical brain regions showing significant volume reductions in DM1 patients compared to healthy controls are represented in blue (FreeSurfer, version 6). (RH: right hemisphere; LH: left hemisphere)

The most significant correlations (p-value<0.01) between neuroimaging, clinical and neuropsychological data are reported in Table 65.

As concerns the correlation of clinical data with subcortical regions and ventricles, negative correlations were found between left and right putamen nuclei reduction and O_2 saturation levels and for only the left putamen nucleus, the volume reduction positively correlated with the percentage of oxygen level (EGA_p02).

MIRS total scores showed negative correlations with left and right putamen nuclei reduction and the decrease volume of corpus callosum mid anterior part.

Left lateral ventricle enlargement negatively correlated with performance on short-term verbal memory task (Digit span forward), while both left lateral and third ventricles enlargement negatively correlated with emotion attribution abilities in a social cognition task (SET task, EA subtask).

At the cortical level, the volume of right superior temporal sulcus's banks positively correlated with nonverbal reasoning scores at CPM-47 test. Positive correlations were also found between left lateral occipital volume and performance on visuoperceptive (BJLOT) and long-term visuospatial memory tasks (ROCF differed recall).

Right middle temporal volume was positively correlated with both visuoconstructional (ROCF copy) and visuoperceptive (BJLOT) abilities, while it negatively correlated with both total score and autonomy in daily activities subitem scores on the ICS questionnaire.

Right superior temporal volume was negatively correlated with executive function abilities (Stroop test).

Left middle temporal volume was positively correlated with respiratory parameters (EGA_p02 and Mean oxygen's saturation levels).

Finally, a negative correlation was found between left supramarginal volume and a sleep apnoea parameter (Index of central apnoea/hours of sleep).

Table 64. Neuropsychological and psychological performances in DM1 patients and comparison between E1 and E2 subgroups

Neuropsychological and psychological assessments													
		All DM1				E1			E2				
Cognitive and psychological domain	Tests	Score (mean)	sd	normal values cut offs	% pathological scores	Score (mean)	sd	% pathological scores	Score (mean)	sd	% pathological scores	F value	P-value
Cognitive screening	MMSE score	27.44	2.71	≥23,8	8.57%	28.36	1.01	0%	27.33	2.82	14.29%	1.82	0.1871
Non-verbal intelligence	CPM-47	28.83	6.93	≥18,96	14.29%	31.71	3.75	0%	28.33	6.97	23.81%	3.23	0.0820
Verbal short-term memory	Digit Span Forward	5.25	1.16	≥3,75	25.71%	5.36	1.22	14.29%	5.19	1.21	33.33%	0.16	0.6950
	BSRT_immediate recall	5.79	1.70	≥3,87	11.43%	5.89	1.77	14.29%	6.06	1.22	9.52%	0.13	0.7262
	RAVL_immediate recall	51.83	10.29	≥28,53	8.57%	52.36	9.84	7.14%	51.57	11.38	9.52%	0.05	0.8263
Visuospatial short-term memory	Corsi's Block Test	4.75	1.27	≥3,75	31.43%	5.21	1.05	21.43%	4.67	1.28	38.10%	1.94	0.1739
Verbal long-term memory	BSRT_delayed recall	5.40	2.05	≥3,77	22.86%	5.69	1.91	21.43%	5.47	2.02	23.81%	0.16	0.6917
	RAVL_delayed recall	11.81	2.94	≥4,69	8.57%	11.64	2.71	7.14%	11.81	3.06	9.52%	0.04	0.8449
Visuospatial long-term	ROCF_delayed recall	13.40	7.17	≥9,47	57.14%	15.75	7.14	35.71%	12.07	7.05	71.43%	2.73	0.1088
Language	BNT-short version	26.64	3.31	≥22	8.57%	27.36	1.98	0%	26.24	3.79	14.29%	1.24	0.2743
	Associative Fluency Test	39.25	15.38	>17,35	22.86%	40.93	13.18	14.29%	40.10	16.58	28.57%	0.03	0.8688
	Category Words Fluency Test	49.67	11.84	≥25	8.57%	50.36	10.77	7.14%	49.76	12.76	9.52%	0.02	0.8807
Executive functions	FAB	14.56	3.06	≥13,5	48.57%	15.21	2.55	42.86%	14.33	3.25	52.38%	0.95	0.3371
	Stroop test (RT-sec)	26.06	20.91	>17,35	34.29%	23.25	13.94	42.86%	27.12	23.52	28.57%	0.36	0.5530
	Digit Span Backward	4.08	1.18	≥2,65	28.57%	4.29	1.14	21.43%	4.10	1.14	33.33%	0.24	0.6250
Attention	Attentional Matrices test	49.36	8.44	≥31	25.71%	53.21	6.18	14.29%	48.05	8.87	33.33%	3.97	0.0552
	SDMT	43.67	15.44	≥34,20	40.00%	41.21	10.62	42.86%	44.95	17.10	38.10%	0.75	0.3931
Visuoconstructional abilities	ROCF_copy	27.28	8.87	≥ 28,88	51.43%	30.86	7.26	21.43%	26.71	8.45	71.43%	3.02	0.0920
Visuoperception	SCT	8.86	3.12	≥2,25	17.14%	7.64	2.21	28.57%	9.95	3.20	14.29%	5.94	<.05*
	BJLOT	20.72	8.00	≥18	28.57%	23.50	6.27	14.29%	20.38	7.70	38.10%	1.67	0.2058
Social cognition	Eyes Mind test	20.86	4.11	≥14	8.57%	22.86	2.25	0%	19.86	4.52	14.29%	6.90	<.05*
	SET Total score			No cut-offs		15.57	1.55	*N.A*	16.95	1.50	*N.A*	9.09	<.005**
	SET EA score					5.36	0.93	*N.A*	5.71	0.56	*N.A*	2.31	0.1390
	SET IA score					5.29	0.91	*N.A*	5.71	0.64	*N.A*	5.11	<.05*
	SET CI score					4.93	0.92	*N.A*	5.52	0.68	*N.A*	5.60	<.05*
Anosognosia	MAI	-1.12	2.09	>-2	11.43%	-1.00	1.91	7.69%	-0.95	2.48	15.00%	0.00	0.9507
Mood	BDI-II	10.06	7.82	≤12	28.57%	7.08	5.82	15.38%	11.45	8.15	40.00%	2.74	0.1080
	STAI-Y_State score	37.88	8.54	≤40	40.00%	35.62	7.70	38.46%	39.35	8.03	45.00%	1.70	0.2020
	STAI-Y_Trait score	39.32	9.99	≤40	45.71%	34.08	8.76	30.77%	42.05	10.03	60.00%	5.20	<.05*
	STAXI-II_State score	54.94	19.89	≤75°	20.00%	10.62	1.45	7.69%	12.25	3.13	30.00%	3.30	0.0795
	STAXI-II_Trait score	67.21	22.40	≤75°	42.86%	19.15	4.20	30.77%	22.10	3.80	55.00%	4.17	0.0504
	SAS	14.73	5.05	≤14	48.57%	11.77	4.13	30.77%	15.68	4.60	68.42%	5.73	<.05*
Social behaviour	ICS	5.74	7.32	No cut-offs		5.55	5.63	*N.A*	5.11	7.50	*N.A*	0.04	0.8484
Cognitive reserve	CRIq_total score	102.00	*11.77*	No cut-offs		111.14	11.67	*N.A*	98.71	8.94	*N.A*	20.73	<.001***
	CRIq_E	102.64	*10.51*			107.93	12.52	*N.A*	101.67	10.14	*N.A*	10.23	<.005**
	CRIq_WA	104.31	*11.51*			111.00	12.08	*N.A*	101.24	10.11	*N.A*	9.64	<.005**
	CRIq_LT	97.28	14.25			106.07	12.95	*N.A*	93.86	11.58	*N.A*	8.30	<.05*
Qually of Life	SF-36_GH	49.85	*20.36*	≥56.99	20.00%	54.23	18.69	7.69%	46.75	22.38	30.00%	1.17	0.2893
	SF-36_PF	67.35	*25.11*	≥70.61	*34.29%*	78.46	25.53	15.38%	64.00	21.00	50.00%	3.23	0.0830
	SF-36_PH	72.06	*34.69*	≥52.97	*17.14%*	88.46	19.41	15.38%	65.00	36.63	20.00%	4.38	<.05*
	SF-36_P	69.98	*25.81*	≥70.77	*45.71%*	71.35	30.97	46.15%	71.46	20.71	50.00%	0.00	0.9900
	SF-36_EP	71.76	*32.00*	≥65.78	*22.86%*	87.18	21.68	7.69%	63.66	34.24	35.00%	5.10	<.05*
	SF-36_E/F	48.09	*19.42*	≥52.15	*40.00%*	56.54	14.20	15.38%	45.25	21.91	60.00%	2.65	0.1150
	SF-36_EWB	67.99	*17.06*	≥70.38	*17.14%*	75.38	13.74	7.69%	62.77	17.55	25.00%	4.62	<.05*
	SF-36_SF	*68.16*	*21.03*	≥78.77	*31.43%*	73.08	23.85	38.46%	65.88	19.22	30.00%	0.87	0.3590
	SF-36_HC	*41.32*	15.04	≥59.14	*91.43%*	40.38	12.66	100.00%	44.00	16.11	85.00%	0.44	0.5140

SD: standard deviation; N.A: not available; BSRT: Babcock Story Recall Test; RAVL: Rey Auditory Verbal Learning Test; CPM 47: Raven's Coloured Progressive Matrices; ROCF: Rey-Osterrieth complex figure; BNT-short version: The Boston Naming Test short 30-item version; FAB: Frontal Assessment Battery**;** RT-sec**:** Reaction time, seconds; SDMT: Symbol Digit Modalities Test; B JLOT: Benton Judgment of line orientation test-h version; Eyes Mind test: Reading the Mind in the Eyes'test; SET: Story-based Empathy Task; SET-EA: Emotion attribution score; SET-IA: Intention attribution score; SET-CI: Causal Inference score; BDI-II: Beck Depression Inventory, 2nd version; STAI-Y: State-Trait Anxiety Inventory; STAXI-II: State-Trait Anger Expression Inventory; CRIq: Cognitive Reserve Index questionnaire; CRIq_E: CRI_education score; CRIq_WA: CRI_working activity score; CRIq_LT: CRI_Leisure-Time score; SF-36_GH: General health; SF-36_PH: Physical health; SF-36_PF: Physical functioning; SF-36_P: Pain; SF-36_EP: Limit due to emotional problems; SF-36_E/F: Energy.fatigue; SF-36_EWB: Emotional wellbeing; SF-36_SF: Social functioning; SF-36_ Health change; SAS: Starkstein Apathy Scale; MAI: Measurement of Anosognosia Instrument; SCT: Street's completion test; ICS: Intervista sul comportamento spontaneo

Table 65. Brain volumetry and correlations analysis results.

Subcortical regions and ventricles																
Brain region	F value [F(2,60)]	P value	Post-hoc		E1		E2		HC		Correlations with NPS data			Correlations with clinical data		
			contrast	p-value	mean (mm3)	sd (mm3)	mean (mm3)	sd (mm3)	mean (mm3)	sd (mm3)	Measure	p-value	r	Measure	p-value	r
Right Accumbens	11.45	0.0000612	E2-E1	0.84843	452	107	468	85	568	92						
			HC-E1	0.00013												
			HC-E2	0.00013												
Left Accumbens	11.02	0.0000839	E2-E1	0.89506	422	104	435	108	535	82						
			HC-E1	0.00022												
			HC-E2	0.00016												
Right Putamen	19.44	0.0000003	E2-E1	0.35136	4118	371	3902	513	4733	667				MIRS_T3_Score	0.007	-0.570
			HC-E1	0.00018										T3.ODI	0.003	-0.606
			HC-E2	<E-10												
Left Putamen	16.03	0.0000026	E2-E1	0.34597	4131	459	3899	471	4723	668				MIRS_T3_Score	0.001	-0.656
			HC-E1	0.00075										EGA_pO2_t3	0.003	0.603
			HC-E2	<E-10										T3.ODI	0.005	-0.585
Corpus Callosum Mid Posterior	8.87	0.0004211	E2-E1	0.96856	462	115	470	89	568	78						
			HC-E1	0.00128												
			HC-E2	0.00053												
Corpus Callosum Mid Anterior	9.10	0.0003545	E2-E1	0.33958	480	157	425	99	562	96				MIRS_T3_Score	0.001	-0.657
			HC-E1	0.06514												
			HC-E2	0.00012												
Right Lateral Ventricle	14.91	0.0000055	E2-E1	0.37445	16313	12634	13549	6615	7164	3037						
			HC-E1	0.00003												
			HC-E2	0.00067												
Left Lateral Ventricle	10.33	0.0001393	E2-E1	0.10159	19647	16246	13895	6908	8244	2780	Digit span forward	0.008*	-0.594			
			HC-E1	0.00009							SET_EA	0.004**	-0.624			
			HC-E2	0.03300												
3rd Ventricle	20.42	0.0000002	E2-E1	0.02348	2005	916	1590	614	1111	396	SET_EA	0.008*	-0.591			
			HC-E1	<E-10												
			HC-E2	0.00063												
Cortical regions																
Brain region	F value [F(2,60)]	P value	Post-hoc		E1		E2		HC		Correlations with NPS data			Correlations with clinical data		
			contrast	p-value	mean (mm3)	sd (mm3)	mean (mm3)	sd (mm3)	mean (mm3)	sd (mm3)	Measure	p-value	r	Measure	p-value	r
Right banksst	12.11	0.0000383	E2-E1	0.60756	2000	313	1905	327	2342	312	CPM 47	0.007*	0.602			
			HC-E1	0.00128												
			HC-E2	0.00000												
Left fusiform	12.10	0.0000384	E2-E1	0.98383	9049	1154	9105	1526	10400	1062						
			HC-E1	0.00007												
			HC-E2	0.00001												
Right isthmus cingulate	9.92	0.0001902	E2-E1	0.00847	2476	264	2124	437	2557	374						
			HC-E1	0.72582												
			HC-E2	0.00004												
Left lateral occipital	9.60	0.0002423	E2-E1	0.88830	11150	1698	10917	2026	12813	1536	ROCF differed recall	0.003**	0.633			
			HC-E1	0.00183							BJLOT	0.003**	0.636			
			HC-E2	0.00004												
Left lateral-orbito frontal	10.95	0.0000881	E2-E1	0.88006	6990	831	6893	1052	7771	648						
			HC-E1	0.00026												
			HC-E2	0.00000												
Right middle temporal	12.95	0.0000212	E2-E1	0.87386	10557	1457	10746	1318	12265	1322	ROCF copy	0.003**	0.634			
			HC-E1	0.00002							BJLOT	0.003**	0.632			
			HC-E2	0.00001							ICS_AQ	0.001***	-0.684			
											ICS_Total	0.001***	-0.681			
Left middle temporal	9.45	0.0002711	E2-E1	0.93415	9846	1587	9992	1916	11442	1119				EGA_pO2_t3	0.003**	0.609
			HC-E1	0.00026										T3.mean nocturnal Sa02	0.002**	0.621
			HC-E2	0.00013												
Left pars opercularis	9.75	0.0002162	E2-E1	0.98605	4007	542	4043	682	4829	753						
			HC-E1	0.00050												
			HC-E2	0.00012												
Right precentral	9.65	0.0002324	E2-E1	0.62754	11420	1639	10959	1935	13011	1781						
			HC-E1	0.00273												
			HC-E2	0.00001												
Left precentral	9.12	0.0003486	E2-E1	0.50246	11891	1567	11403	1740	13207	1354						
			HC-E1	0.00460												
			HC-E2	0.00001												
Right precuneus	14.04	0.0000100	E2-E1	0.16351	9225	1657	8628	1513	10399	1043						
			HC-E1	0.00061												
			HC-E2	<E-10												
Left precuneus	10.57	0.0001164	E2-E1	0.10984	8948	1476	8314	1566	9855	922						
			HC-E1	0.00636												
			HC-E2	0.00000												
Left rostral-middle frontal	9.48	0.0002652	E2-E1	0.91767	12774	1814	12995	2782	15039	2130						
			HC-E1	0.00012												
			HC-E2	0.00006												
Left superior frontal	9.48	0.0002652	E2-E1	0.23320	18666	3125	17506	3016	21109	2580						
			HC-E1	0.00104												
			HC-E2	0.00000												
Right superior parietal	9.85	0.0002005	E2-E1	0.06508	12110	2426	11028	2201	13258	1401						
			HC-E1	0.02797												
			HC-E2	0.00000												
Right superior temporal	14.30	0.0000083	E2-E1	0.07119	10678	1523	9930	1538	11701	1118	Stroop test_RT	0.009*	-0.586			
			HC-E1	0.00399												
			HC-E2	<E-10												
Right supramarginal	14.30	0.0000083	E2-E1	0.41554	9122	1709	8651	1749	10493	1088						
			HC-E1	0.00046												
			HC-E2	0.00000												
Left supramarginal	16.25	0.0000023	E2-E1	0.87354	9566	2034	9347	1679	11475	1235				T3.Central apnoea index	0.005**	-0.591
			HC-E1	0.00004												
			HC-E2	0.00000												
Right transverse temporal	12.59	0.0000272	E2-E1	0.01354	878	239	737	143	971	160						
			HC-E1	0.09490												
			HC-E2	0.00000												
Left transverse temporal	14.67	0.0000065	E2-E1	0.41048	1020	246	940	185	1244	199						
			HC-E1	0.00071												
			HC-E2	0.00000												

SD: standard deviation; *: p<.05; **: p< .005; ***: p<.001; CPM 47: Raven's Coloured Progressive Matrices; ROCF: Rey-Osterrieth complex figure; BJLOT: Benton Judgment of line orientation test-h version; ICS_AQ: Intervista sul comportamento spontaneo_subscore Attività quotidiane; ICS_AQ: Intervista sul comportamento spontaneo_Total score; RT-sec**:** Reaction time, seconds; SET-EA: Story-based Empathy Task_Emotion attribution score; MIRS: Muscular Impairment Rating Scale

DISCUSSION

Myotonic dystrophies (DMs) are progressive autosomal dominant, multisystem diseases with a core pattern of clinical presentation including muscular involvement (skeletal and smooth muscle) with myotonia and dystrophy, cardiac conduction defects, respiratory involvement, ocular disturbances, endocrine and gastrointestinal disorders; moreover, central nervous system involvement (sleep disorders, cognitive impairment, behavioural and psychiatric disorders) is now widely recognized and peripheral nerve involvement has also been described. Both DM1 and DM2 are caused by an abnormal expansion of an unstable nucleotide repeat located in the non-coding region of their respective genes DMPK for DM1 and CNBP in DM2. Mutant transcripts contain the triplet repeats form RNA hairpins that accumulate as foci in cell nuclei; these toxic transcripts are thought to sequester alternative splicing regulators leading to splicing defects in various genes that are considered the primary cause of the multisystem involvement of the disease. Moreover, repeat mutations are dynamic gene defects that show instability: different numbers of repeats in different tissues (somatic mosaicism) increase over time in the same individual and across generations (the so-called "anticipation phenomenon" in which disease severity increases and age of onset decreases from one generation to the next); the dynamic nature of the mutation makes genotype-phenotype correlation difficult and may explain, at least in part, the high variability of muscular and extra-muscular symptoms, often causing a delay in diagnosis (1, 4, 5). Due to the rarity of the disease, there are currently no prospective studies conducted on large numbers of patients with a complete phenotypic and genetic characterization.

Myotonic dystrophy type 1 (or Steinert disease) is the most common inherited muscular dystrophy in adults with an estimated prevalence of 1/8000. Patients with DM1 can be divided into five main categories, based on age at onset and severity of symptoms, each presenting specific clinical features and management problems: congenital, childhood-onset, juvenile, adult-onset, and late onset/asymptomatic (8, 5).

Regarding the size of CTG expansion, patients are divided into 3 groups: E1 (characterized by 50-150 repeats CTG, often with a mild clinical phenotype), E2 (150-1000 repeats CTG, usually with a classic phenotype with a wide span from mild to severe symptoms) and E3 class of expansion (characterized by > 1000 repeats CTG mainly associated with the congenital form). In DM1 patients, the repeat expansion length is predictive of clinical severity and age of onset, however, due to somatic mosaicism, CTG repeat size correlates more significantly with age of onset and disease severity below 400 CTG repeats (4).

In the present work we described a cohort of 72 DM1 (24 E1, 41 E2 and 7 E3) patients with the main objective of a broad and complete phenotypic characterization and analyses of possible genotype-phenotype correlations.

1. ANAMNESTIC AND NEUROLOGICAL CHARACTERISTICS

About one third of patients reported a disease onset in adulthood, a quarter of patients a late onset form, followed by juvenile and congenital/childhood onset. The E3 size of expansion predominated in the congenital onset group of patients, the E2 among the juvenile, while the E1 predominated among adult and late onset group of patients. The results of our study confirm what is already known in literature about the pre-eminently maternal transmission of congenital forms. Furthermore, we confirm the correlation between the CTG repeat length (measured on circulating leukocytes), the age of onset and the severity of the disease. In fact, we found an earlier age at onset and a greater frequency of perinatal problems and delayed psychomotor development in the E3 group of patients.

Neurological examination confirmed, at the onset of disease, a greater involvement of distal limb muscles.

We found no statistically significant differences in terms of muscle involvement between the different classes, except for the involvement of facial muscles in class E3.

Among extra-muscular symptoms at onset, excessive daytime sleepiness was present in about 20% and fatigue in about 30% of patients.

2. CARDIOLOGICAL EVALUATION

The early stages of cardiac involvement in MD are typically clinically silent. Phenotypic variability results in a wide spectrum of clinical manifestations even amongst members of the same family (97). The most common cardiac manifestations of MD are arrhythmic: there is an increased risk for conduction abnormalities, atrial arrhythmias (atrial flutter and fibrillation) and ventricular arrhythmias; cardiomyopathy (especially dilatative) and, less frequently, coronary heart disease have also been described (97). Cardiac arrhythmias can be asymptomatic and can precede the onset of neuromuscular symptoms, therefore regular cardiological screening is recommended in DM1 patients (88). Correlations between CTG expansion size and cardiological manifestations have not been universally observed; some studies described a correlation especially with conduction disturbances (94-96, 97-98, 99-107), while other authors did not confirm this association (108-112). Cardiac abnormalities appear to be more consistently associated with age, duration of neurological disease and male sex than with CTG repeats (113, 97, 22). Moreover, cardiological involvement can be worsened by concomitant respiratory failure and apnoea, but only few studies examined cardiological and pulmonary parameters correlations in DM1 patients (120-122, 107).

In our sample we found a cardiological involvement in about two thirds of patients, with a prevalence of conduction disorders, confirming previous studies; we did not find significant differences among the 3 genetic classes of expansion. We found that disease duration was associated with longer ECG PR length and lower ejection fraction on echocardiogram, suggesting a progressive cardiological involvement in DM1 patients. Comparing patients with cardiopathy (defined as ejection fraction $\leq$ 50%) and without cardiopathy we observed that patients with cardiopathy had a longer disease duration, a higher BMI, lower values of pulmonary function parameters forced vital capacity (CVF) and forced expiratory volume in the first second (FEV1), lower maximum heart rate, higher obstructive apnoea/hypopnoea, and mixed apnoea index. In the adjusted

logistic regression, higher CVF values were significant predictors for a reduced risk of cardiopathy, while FEV1 and obstructive apnoea/hypopnoea/mixed apnoea index were no longer associated with cardiopathy. In line with our results, other authors found that restrictive syndrome (CVF < 80% than predicted) was an independent prognostic factor of cardiac events in a multivariate Cox analysis (107).

Comparing patients with conduction disorder (defined as PR interval length ≥200 msec, QRS ≥100 msec) and without conduction disorder, we found that the first group of patients had longer disease duration and higher MIRS score, and these associations were confirmed after applying logistic regression; on the contrary, the associations between conduction disorder and lower ejection fraction, lower nocturnal saturation values, higher oxygen desaturation index (ODI) and obstructive apnoea/hypopnoea/mixed apnoea index, were not confirmed by logistic regression.

Taken together our results suggest that cardiological involvement is progressive and can be closely associated with the progression of pulmonary and muscle involvement; however, the lack of significant differences among CTG expansion and the observation that most patients can be asymptomatic, confirm the importance of a close cardiological follow-up of these patients.

3. RESPIRATORY EVALUATION

In DM1 patients, mortality is mainly due to respiratory problems, usually resulting from respiratory failure or aspiration. The predominant respiratory function abnormality in these patients is a restrictive ventilatory pattern resulting in a reduction of maximal inspiratory pressure (MIP), maximal expiratory pressure (MEP), forced vital capacity (CVF) and forced expiratory volume in the first second (FEV1); over time chronic respiratory failure develops with hypoxemia (defined as arterial oxygen partial pressure PaO2 < 80 mmHg) and hypercapnia (defined as partial carbon dioxide pressure PaCO2 >45 mmHg) (105). There is no consensus in literature regarding the relationship between CTG repeat length and severity of respiratory dysfunction; a relationship was observed in some studies (122, 127, 136, 138-140), but not in others (141, 142, 144); abnormal respiratory function tests were found to be associated with severity of muscle disease (122, 135, 136, 138, 139, 107, 142), BMI (127, 140, 136, 138), duration of muscle symptoms (138), cardiac conduction abnormalities (107, 136, 122) and hypoxaemia (127). Regarding arterial blood gas parameters, the association between hypercapnia and CTG repeat length was found only in one study (127) while others found no clear associations (105); only some studies found a correlation between hypercapnia and spirometry parameters, while most studies did not confirm this association (127, 130, 141, 142, 146, 148). The observation of a relative preservation of CVF in hypercapnic patients suggested that a central ventilatory control dysfunction can be responsible for breathing involvement in these patients, in association with respiratory muscle weakness and myotonia. Some studies pointed out that an abnormal sensitivity of the central respiratory drive to chemical blood changes, particularly of (Co2), might contribute to the pathogenesis of respiratory impairment in DM1 (127); the same authors found that the supine fall in FEV1 was the only variable associated with ventilatory restriction, hypoxaemia and hypercapnia (133). The hypothesis of a central dysfunction could be supported by

findings of severe neuronal loss in various medullary nuclei linked to respiratory function (105, 127, 129, 130, 131, 132, 203).

In agreement with the literature, we found that respiratory dysfunction, predominantly a restrictive ventilatory pattern, is common in DM1 patients and is associated with alveolar hypoventilation and chronic hypercapnia. We found a significant correlation between class of expansion and functional pulmonary parameters (CVF, FEV1, MIP and MEP) that showed a significant reduction in E3 class of expansion compared to E2 and E1 patients. However, we did not find significant differences among the 3 groups of patients analyzing the arterial blood gas values. Similar results were obtained when comparing patients stratified by age at onset.

Moreover, we observed a significant reduction of CVF, FEV1 and MEP with disease duration and a parallel worsening in Muscular Impairment Rating Scale (MIRS) confirming the results of previous studies (122, 138, 150); in addition, we found a similar association with the Neuromuscular Impairment Function and Disability Scale (NIFDS) score. The more marked impairment of MEP could reflect the weakness of abdominal muscles, which are the major expiratory muscles and that usually have more weakness compared to inspiratory muscles, as previously suggested (133). On the contrary, arterial blood gas parameters did not show significant correlation with disease duration.

Comparing patients with restrictive syndrome (defined as CVF<80%) and patients without restrictive syndrome, we found that the first had higher BMI and disability score (measured by MIRS and NIFDS scale), longer PR interval length on ECG, higher ODI index and obstructive apnoea/hypopnoea and mixed apnoea index, higher AHI index, and lower nocturnal SaO2 values; in the adjusted logistic regression, higher MIRS values were significant predictors for an increased risk of restrictive syndrome, while PR and BMI were no longer associated with this outcome. No statistically significant differences regarding diurnal arterial blood gas parameters were found among the two groups of patients, suggesting that these parameters can be, at least in part, independent of the progressive muscular involvement as previously observed by other authors (127, 130, 141, 142, 146, 148).

Moreover, we found that patients with hypercapnia (PaCO2 ≥45 mmHg) had lower SaO2 and pO2 values, while no differences were found with respiratory function test or MIRS score when compared to patients without hypercapnia; on the univariate analysis we also found higher score in myotonia subitem of the NIFDS scale in hypercapnic patients, but after applying the adjusted logistic regression this association was not confirmed. Similarly, patients with hypoxemia (PaO2 < 80 mmHg) had higher pCO2 values, but no significant differences in respiratory function test or MIRS score when compared to patients without hypoxemia. However, using the continuous variable PaO2 we found a positive association with CVF and negative correlations with NIFDS total score and daily life domain of the scale; in the adjusted logistic regression higher CVF values were significant predictors for a reduced risk of hypoxemia.

Our results are in agreement with previous studies, confirming that arterial blood gas values are at least in part independent of the grade of muscular impairment, suggesting a possible central nervous system (CNS) dysfunction in DM1 patients with respiratory failure (105, 122, 127, 130). In a recent study, Mazzoli et al observed a different behaviour of CVF, pO2 and pCO2 values in the three genotypes: while in E3 patients

there was a relationship between hypercapnia and pathological values of CVF (suggesting a dysfunction of respiratory muscles), in genotype E2 slight hypercapnia was associated with CVF values within normal ranges, supporting the hypothesis that in this group of patients alveolar hypoventilation could be due to dysregulation of the central drive (122).

On the other hand, the trend of respiratory function test (and in particular CVF and FEV1) seems to be significantly associated with clinical muscle severity; to confirm this hypothesis we observed that patients with higher MIRS score had lower values of FEV1, CVF and MEP, as well as lower age at onset, longer disease duration, higher score in NIFDS scale. However, a recent study found that greater CTG repeat size, higher MIRS rating, and longer disease duration were all correlated with lower baseline CVF, but not with annual rate of change, suggesting that decline is not due to progressive muscle impairment alone; authors explored also the impact of NIV compliance on the rate of decline of CVF and found that NIV compliant patients experiences slower rates of FVC decline compared to non-compliant patients, supporting the utility of NIV (138).

4. SLEEP DISORDERS

Central Nervous System (CNS) dysfunction, in particular sleep disorders and cognitive impairment, represents one of the major issues affecting quality of life in DM1 patients.

DM1 is associated with a high prevalence of sleep disorders, predominantly excessive daytime sleepiness (EDS) and sleep related breathing disorders (SRBD) and particularly sleep apnoea. Persistent nocturnal hypoxemia can result in cardiovascular and pulmonary failure; in addition, sleep fragmentation and excessive daytime sleepiness lead to disability and may affect mood and cognition.

EDS is one of the most frequent non-muscular symptoms in DM1 patients, appearing in up to 70–80% of DM1 patients (203); unlike in narcolepsy, in DM1 patients EDS is characterized by persistent sleepiness unaffected by naps, the latter being frequently long, unrefreshing, and without dream content.

The pathogenesis of EDS in DM1 patients remains unclear. The hypothesis of a dysfunction of the hypothalamic hypocretin system was suggested by Martınez-Rodrıguez et al. (193) but was not confirmed by more recent studies (187, 204). Some authors observed that DM1-related EDS was associated with obstructive, central sleep apnoea and/or hypoventilation (333, 334). However, most studies did not find any association between EDS, age, gender, BMI, CTG repeat number, daytime pulmonary function test or arterial blood gas parameters, MSLT results or with polysomnography (PSG) findings including periodic limb movement disorders (PLMD), REM sleep characteristics, nocturnal respiratory events and mean O2 desaturation (187-190, 195, 193, 202, 203, 144). The observation that EDS can occur in the absence of a significant breathing disorder or even before the onset of muscle symptoms suggests a possible dysfunction of brain areas involved in the regulation of the sleep–wake cycle (189, 196). Indeed, post-mortem studies of DM patients showed reduced serotoninergic and catecholaminergic neurons, respectively, in the dorsal raphe and superior central nuclei and in the medullary reticula formation (132, 331).

In our study, using the Epworth Sleepiness Scale (ESS), we found pathological daytime sleepiness (defined as ESS score ≥ 10) in 16.3% of patients, without significant differences among the 3 genetic classes. In agreement with previous studies (188-190), comparing patients with excessive daytime sleepiness and patients without excessive daytime sleepiness (ESS <10), we found no associations with age, disease duration, gender, BMI, MIRS scale, arterial blood gas values, oxygen desaturation index (ODI) or apnoea/hypopnoea index, nor with cardiological parameters. In addition, we found positive correlations among ESS score, neuropsychological domain (NP) of the NIFDS scale and COMPosite Autonomic Symptom (COMPASS 31) total score and its subitems (secretomotor, gastrointestinal and pupillomotor area), suggesting a possible greater disease burden in these patients. The positive association with the NP domain of the NIFDS could suggest that DM1 patients with EDS are more prone to psychological distress, as previously observed (335-337).

Regarding sleep related breathing disorders (SRBD), recent case control studies found that DM1 patients had higher apnoea index, central apnoea index, and apnoea hypopnoea index (AHI) during NREM sleep (188, 190). The percentage of patients with apnoea varies among studies from 30% to 86% of patients with a prevalence of obstructive events (190, 202, 144, 189). It has been observed that sleep apnoea symptoms, including EDS, morning headaches, frequent arousals, tiredness or dyspnoea may be absent or minimal despite significant apnoea and severe nocturnal oxygen desaturation (202); because polysomnography (PSG) is expensive and not universally available, it would be important to identify possible daytime predictors of SRBD.

Previous studies investigated the relationship between daytime respiratory function and sleep abnormalities, with conflicting results (105, 148, 144, 189, 202). Kian et al observed that both CVF and FEV1 were moderately correlated with daytime arterial oxygen saturation (SaO2), with nocturnal oxygen desaturation index (ODI) and apnoea-hypopnoea index (AHI); they also found a moderate correlation between daytime arterial oxygen partial pressure (PaO2), nocturnal SaO2 and ODI index, while daytime arterial carbon dioxide partial pressure (PaCO2) had no correlation with nocturnal parameters (148). Subsequent studies, with larger patient samples, demonstrated that daytime pulmonary function tests, arterial blood gas values, degree of neuromuscular impairment, genetic class, age, sex, BMI, daytime sleepiness, cardiac dysfunction, cannot predict the development of SRBD (202, 189, 144, 105). Laberge et al in a series of 43 DM1 patients found that, although the apnoea-hypopnoea index (AHI) was significantly correlated with total lung capacity and vital capacity, these lung volume measurements accounted for only 16% of AHI variance (189). Overall, these authors agree that DM1 patients may exhibit clinically significant SRBD even in the presence of normal daytime pulmonary function, supporting the hypothesis of a central control of ventilation dysfunction.

In agreement with literature, in our study sleep apnoea was detected in a large percentage of DM1 patients, with no significant differences between the 3 genetic classes regarding the type and severity of apnoea; there was a predominance of pure obstructive apnoea, even if about one third of patients presented both obstructive/central apnoea and some patients had pure central apnoea.

We observed that patients with sleep apnoea had a higher BMI and lower CVF values than patients without sleep apnoea, while no differences were detected regarding age at onset, disease duration, disability scales, arterial blood gas values or cardiological parameters. In the adjusted logistic regression, higher CVF values were significant predictors for a reduced risk of apnoea.

Moreover, the nocturnal oxygen desaturation index (ODI) showed significant negative associations with CVF, FEV1 and diurnal pO2, and positive association with the daily life activity (DL) domain and total score of the NIFDS scale; all these associations were confirmed in the adjusted linear regression. On the other hand, the positive associations with MIRS, PR and QRS interval length that we found on the univariate analysis were not confirmed after linear regression.

Similarly, we also observed that obstructive AHI and mixed apnoea index had negative correlations with CVF and FEV1 and a positive association with DL score and PR interval length on ECG, and all these correlations were confirmed after applying linear regression; on the contrary, the positive association with MIRS and QRS on univariate analysis were no longer significant after correction.

Regarding nocturnal saturation parameters, we found that minimal SaO2 and mean SaO2 had significant positive correlations with diurnal pulmonary parameters CVF, FEV1 and pO2; in addition, mean SaO2 showed positive correlation with heart rate and diurnal SaO2. These associations were all confirmed in the adjusted linear regression, while the negative association between min SaO2 and MIRS score was not confirmed.

Overall, our results seem to suggest that at least some diurnal parameters could identify patients at lower risk of sleep related breathing disorders. In particular, higher CVF values seem to be significant predictors for a reduced risk of apnoea, as confirmed also by the observation that CVF, FEV1 and pO2 values were all associated with lower ODI and AHI index and higher nocturnal saturation parameters. On the other hand, the positive association between ODI index and obstructive AHI/mixed apnoea index with daily life activity and total score of the NIFDS scale, and the positive association between obstructive AHI/mixed apnoea index with PR interval length, could suggest that patients with more advanced disease and higher disease burden are also at higher risk of SRBD. However, in patients with intermediate levels of disease burden, a nocturnal respiratory event seems to be, above all, unpredictable and at least in part independent of the severity of muscular impairment, as suggested by the lack of association with MIRS scale and class of expansion. This observation underlines the importance of studying all DM1 patients with periodic cardiorespiratory monitoring in order to early diagnose SRBD and treat it promptly. The early treatment of these disorders is fundamental to prevent cardiovascular and cognitive consequences and ultimately improve the quality of life of DM1 patients.

5. SKIN BIOPSY AND PUPILLOMETRY

The results of our study confirm the presence of a predominantly subclinical involvement of the peripheral nerve in DM1 patients (in our sample 13.46% of patients undergoing an EMG study had a polyneuropathy). Moreover, we demonstrated, using skin biopsy, the presence of small fibre neuropathy with a significant

involvement of the somatic fibres but not of the autonomic fibres. In our sample of patients, nobody reported pain with neuropathic features (pain was often reported as myalgia or joint pain). Using the COMPASS 31 score we observed a prevalence of gastrointestinal and sudomotor symptoms (various patients reported the presence of diarrhoea or constipation and excessive sweating), however we did not find significant correlations between intraepidermal somatic nerve fibre density and COMPASS score (and its specific subitems), nor with the other clinical and instrumental parameters. No significant differences were found among the 3 classes of expansion (E1, E2, E3) nor among different groups of age at onset or different groups of MIRS scale.

We observed that males presented a significant lower fibre density in the leg compared to females and that fibre density was lower in the leg compared to the thigh. As a potential limitation of the study, however, it should be emphasized that the number of controls used for the skin biopsy comparison is lower than the number of cases and thus the different sample size can potentially impact on the lack of significance for the autonomic component.

Regarding pupillometry results, DM1 patient showed significantly lower pupil size at baseline and a lower constriction response to light (peak amplitude). Lower pupil size at baseline could point to a possible sympathetic dysfunction, and this is further corroborated by the significant correlation with longer disease duration, lower ejection fraction value and longer QRS interval length on ECG, overall indicating a greater disease burden. Overall, the sympathetic dysfunction suggested by these results and the correlation with cardiological parameters can be further explored by evaluating the autonomic innervation of the heart (using cardiac SPECT MIBG) and, at the same time, the anatomical structural damage of the heart through cardiac MRI.

Regarding peak amplitude values (which can be considered as an index of the parasympathetic pupillary response), we observed that patients had lower constriction values; moreover, higher constriction response values were associated with higher percentage of autonomic fibres innervating arrector pili muscles in the thigh on skin biopsy, while lower constriction response values were found in patients with higher disability score and higher apnoea/hypopnoea index. These results can reflect both a parasympathetic dysfunction or possible myotonia of smooth muscle sphincter of the iris, but must be interpreted with caution and we have to analyze data on pupillary response dynamics in order to better evaluate the possible myotonic component of the PLR.

6. NEUROPSYCHOLOGICAL ASSESSMENT AND NEUROIMAGING STUDY

Increasing evidence indicates that in DM, and in particular in DM1 there is in an involvement of the Central Nervous System (CNS). Behavioural and cognitive changes are described in DM1 patients, even in patients with minimal muscle impairment, with phenotypes that can be highly different depending upon the age of disease onset (218, 219). The most severely affected are congenital and childhood onset forms, associated with intellectual disabilities, speech and language delay and reduced IQ value, while in adult onset DM1 cognitive and neuropsychological findings are usually not as severe. Most neuropsychological studies, in order to exclude the presence of a major cognitive impairment or mental retardation, included only patients

with childhood, juvenile or adulthood form of DM1 (252). Many authors documented a frontal lobe dysfunction (238, 239) with a selective impairment of executive functioning, attention, visuospatial and visuoconstructive abilities and perceptual reasoning and an apparent saving of verbal skills (147, 237, 240-242, 283, 344). Okkersen et al. in a recent review observed in DM1 patients a significantly worse performance in every neuropsychological domain tested, compared to controls, in particular for global cognition, intelligence, visual memory, visuospatial perception, visuoconstruction, psychomotor speed, attention, executive functioning, overall and verbal memory, social cognition (261). Cognitive impairment in DM patients may also be influenced by other DM symptoms like depression, fatigue and increased daytime sleepiness.

It is debated whether CNS dysfunction in DM1 patients is, in its nature, a neurodevelopmental, neurofunctional, and/or neurodegenerative disorder; longitudinal cognitive studies in DM1 patients have shown a decline in several cognitive domains, mainly in visuospatial and visuoconstructive abilities, verbal memory, visual attention and executive functions correlating with age and disease duration, suggesting a possible degenerative brain process (238, 244-247). On the other hand, congenital and childhood DM1 longitudinal studies showed no further significant decline in cognitive abilities suggesting a neurodevelopmental alteration in early life (225).

Besides cognitive impairments, behavioural disorders, distinctive personality traits (240, 336), anosognosia (242), social cognition impairment (251, 252, 345-347, 349), apathy (348) and mood disorders (depression and anxiety) (300) are frequently reported in DM1 patients and they have been associated with a poorer quality of life (239, 240, 242). The pathogenesis of psychological disorders in DM1 is debated: they could arise as a reaction symptom to DM1 clinical manifestations or could be a direct consequence of DM1 pathology affecting the brain.

In our study 33 DM1 patients (13 E1 and 20 E2) underwent a full neuropsychological evaluation and a quantitative structural MRI study. Comparing the performance of all DM1 patients (E1 and E2 classes) to the normal values, only a small percentage of patients scored lower than cut-offs in MMSE and CPM-47 test, confirming the observation that neuropsychological deficits in DM might partially escape commonly applied neuropsychological test batteries (239, 218). However, applying more specific tests, we found that the most affected domains were executive functions and visuoconstructional task (about half of patients), attention, visuospatial, visuoperceptual tasks and visuospatial short-term memory (about one third of patients), followed by language task (in particular associative fluency task), short-term and long-term verbal memory, confirming the results of previous studies (147, 237, 240-243, 344). Moreover, a discrete percentage of patients performed worse than controls in social cognition test and had scored above cut-offs for the presence of anosognosia. Almost half of patients of our sample presented significant levels of apathy and we also found high levels of depression, anxiety and anger. Our results are in line with most data in literature (240, 336, 251, 252, 300, 345-349). Cognitive reserve index (CRI) showed a total score belonging to the mean level of CRI in an age- and sex-matched control population. A high percentage of DM1 patients reported a worsened

quality of life, as previously observed (240, 242), and about half of patients reported a significant level of physical pain and worsened ratio of energy/fatigue in daily activities.

Comparing E1 and E2 patients we found a worse performance of E1 patients in the visuoperceptual ability and social cognition tasks, while E2 patients showed a significantly higher global score of apathy and a significant index of mild trait anxiety, suggesting a higher predisposition to react anxiously to a situation.

All cognitive reserve indices were significantly higher in E1 compared to E2 patients, showing the presence of a higher number of protective factors, such as educational level, occupation and leisure time activities, enhancing the ability to optimize and maximize cognitive performance. E1 patients reported a generally higher level of quality of life in all investigated domains, in particular in the domains of physical health, perceived limits due to emotional problems and emotional well-being.

Only few previous studies analyzed the relationship between neuropsychological deficits and CTG expansion size, prevalently in more severe forms of DM1 (childhood and juvenile form). Douniol et al, investigating the psychiatric and cognitive phenotype in patients with the childhood form, observed that patients with severe visual–spatial construction disability had a significantly longer CTG expansion size, as did those with lower IQ (224). The same authors, in their review on the juvenile form of DM1, reported significant correlations between IQ scores and the CTG repeat number in this group of patients (226). Regarding the adult form, recent publications about the correlation of CTG-repeat size and neuropsychological deficits showed contradictory study results (232-235). Serra et al found negative correlations between n(CTG) size in leukocytes and Social Cognition battery test (252). Modoni et al, in their longitudinal study, found no significant correlation between the progression of cognitive decline and the n(CTG) in leukocytes; moreover, they observed that patients belonging to the E2 group, with the highest mean age, obtained scores lower than E3 patients, with particular regard to both linguistic and executive tasks (247).

Regarding neuroimaging, most conventional magnetic resonance imaging (MRI) studies described in DM1 patients a ventricular enlargement and diffuse brain atrophy affecting the frontal, parietal lobes, middle and upper temporal gyrus, hippocampus, and subcortical grey matter (GM) (brainstem nuclei, striatum, thalamus, nucleus accumbens, ventral diencephalon and cerebellum) (236, 237, 279-281, 282-285, 241, 243, 277, 286). The functional impact of subcortical volume loss is of particular interest, given the roles of these structures in maintaining wakefulness, regulating sleep architecture, and in cognitive processing (276, 285).

Extensive white matter (WM) involvement in all cerebral lobes, cingulum bundle, corpus callosum and brainstem (pons) along middle cerebellar peduncles was also demonstrated; white matter hyperintensities (WML) in DM1 are typically bilateral, asymmetric, and predominantly located in periventricular and subcortical white matter, in frontal and particularly in anterior temporal lobes (ATWML) (240, 235, 236, 243, 286, 283). The natural history of WM and GM changes in DM1 is largely unclear, and longitudinal data on MRI abnormalities are widely missing; systematic longitudinal brain imaging studies on large cohorts of congenital, juvenile- and adult-onset DM1 patients over time and against healthy controls have been recently encouraged (286).

In our study, comparing a group of 33 DM1 patients to healthy control subjects, we observed an enlargement of the lateral ventricular volumes and a decrease of volumes in frontal, parietal, temporal and occipital cortices; at the subcortical level, we found a bilateral decrease volume of accumbens and putamen nuclei, confirming results of previous studies (283, 286). In addition, comparing the 2 subgroups of DM1 patients, we found a more severe volume reduction of the isthmus cingulate, the transverse temporal, the superior parietal and temporal gyri in E2 patients. On the contrary, we did not find significant differences in white matter lesion load between the 2 genetic classes of patients.

In literature only few studies investigated the correlation of CTG repeat length with grey matter (GM) (285, 282, 287, 295, 296). Ota et al. found a negative correlation between CTG repeat length and GM volumes of the bilateral motor area and right prefrontal cortex (287). Serra et al. found significant positive correlations between CTG triplet expansion and cortical thickness in the left postcentral gyrus and in the left primary somatosensory cortex, while negative associations were found with posterior cingulate cortex bilaterally and in the right lingual gyrus (252). Van der Plas et al found negative correlations between CTG repeat length and GM volumes in putamen, occipital grey matter, thalamus, and amygdala (285). In the study of Labayru increased CTG expansion correlated with atrophy of the orbitofrontal area, anterior and posterior cingulate cortex, left sensorimotor areas, right temporoparietal junction and precuneus, visual association areas, thalamus, striatum and subcallosal cortex (295). Minnerop et al observed that larger CTG repeat sizes were associated with more severe white matter affection in several brain regions (236). On the contrary, other authors found no correlation between neuroimaging and CTG repeat length (277, 237). Studies comparing patient groups with different disease onset are very limited and most studies excluded congenital and childhood patients in order to limit confounders associated with the markedly diverse clinical continuum, such as intellectual deficits (261, 285). Franc et al. found reduced GM volumes only in adult-onset DM1 patients, but not in patients with congenital onset (275). Caso et al. comparing juvenile (jDM1) and adult onset DM1 patients (aDM1) found that while aDM1 patients had a severe pattern of GM atrophy and WM tract damage, in jDM1 patients WM abnormalities exceeded GM involvement, suggesting a strong effect of age on GM (237). Zanigni et al. observed, after excluding patients with congenital/childhood onset, unchanged subcortical GM changes while the cortical GM reduction was less pronounced than for the entire group; they hypothesized that WM involvement occurs early and might be developmental, while with respect to GM involvement aging seems to play a role, pointing toward a neurodegenerative component (279).

As concerns correlations between MRI data and neuropsychological assessment, we observed a negative association between left lateral ventricle enlargement and performance on short-term verbal memory task, while both left lateral and third ventricles enlargement negatively correlated with emotion attribution abilities in a social cognition task.

At the cortical level, the volume of right superior temporal sulcus banks positively correlated with nonverbal reasoning scores. Positive correlations were also found between left lateral occipital volume and performance on visuoperceptive and long-term visuospatial memory tasks.

Right middle temporal volume was positively correlated with both visuoconstructional and visuoperceptive abilities, while it negatively correlated with both total score and autonomy in daily activities subitem's scores on the ICS questionnaire. Finally, right superior temporal volume was negatively correlated with executive function abilities.

Previous studies observed a significant correlation between visuospatial deficit and a WM major rate lesion, ventricle enlargement and volume loss in the central and anteromedial corpus callosum, bilateral cingulated isthmus, right lateral occipital and right pericalcarine cortex (283). Correlation analyses in the study of Baldanzi et al. revealed an association between delayed recall of verbal memory test and the volume of left postcentral, left middle, and inferior temporal gyri and left supramarginal gyrus (241).

Executive functions (namely reduced flexibility of thinking) were found to be correlated with atrophy of the left secondary visual cortex (243), while IQ estimate, visuoconstructive and executive neuropsychological scores were found to be negatively associated with both global and regional volume decrease, mainly distributed in the frontal, parietal and subcortical region (295). Caso et al found that the severity of cognitive deficits, in particular memory, executive functions, reasoning and visuospatial impairments, correlated with WM damage, but not with GM atrophy (237).

Regarding behavioural and psychiatric dysfunctions, previous studies observed that personality traits were associated with a severe involvement of the dorsolateral prefrontal cortex, cingulum, medial and lateral parietal regions, occipital and temporal lobes (237). Serra et al found significant positive correlation between the correct attribution of sadness and cortical thickness in the left superior temporal gyrus, in the right inferior frontal gyrus, in the right precentral gyrus, in the right angular gyrus, and in the medial frontal gyrus bilaterally; they also showed negative correlations between performances on the Social Situations Test and cortical thickness in the bilateral precuneus, in the right superior parietal cortex, and in the left lateral temporal and occipital cortex (252). An association between lesions in the frontal, temporal, and insular subcortices and decreased emotional sensitivity to disgust and anger among DM1 patients were also described (347).

Other authors described an association between WM brainstem atrophy at the level of the basal pons and the middle cerebellar peduncles, depression and emotional control, confirming the hypothesis that depression in DM1 has at least in part, a pathomorphological correlate, and it is not only a reactive phenomenon (243, 237, 352).

In addition, in our study we analyzed possible associations between MRI data and the other clinical data collected, in particular with pulmonary function, sleep parameters, severity of muscular involvement and cardiological parameters, in order to assess a possible CNS involvement in regulating these functions.

We observed that left middle temporal volume was positively correlated with respiratory parameters (with diurnal arterial oxygen partial pressure pO2 and with nocturnal mean oxygen's saturation levels), while a negative correlation was found between left supramarginal volume and a sleep apnoea parameter (central apnoea index). At a subcortical level we found a negative correlation between left and right putamen nuclei volume reduction and O2 saturation levels and a positive correlation between volume reduction of left putamen nucleus and diurnal arterial oxygen partial pressure pO2. Only few studies in literature assessed the

relationship between neuroimaging, respiratory and sleep parameters in DM1 patients. Van der Plas et al (285) observed larger volume of amygdala and hippocampus, suggesting a possible association with sleep breathing disorders; however, in this study respiratory function was not assessed. In line with this observation, some authors observed both regional increases and decreases in hippocampus volume in patients with sleep apnoea and a possible functional link between stimulation of the amygdala and apnoea (329, 330). Cabada et al. described an association between daytime hypersomnia and volume loss in the right pallidum and right ventral diencephalon (283). Caso and colleagues described an association between sleepiness and brain stem abnormalities (237).

Finally, we found a negative correlation between MIRS total scores and decreased volume of left and right putamen nuclei and corpus callosum mid anterior part. Some authors described a correlation between increased muscular impairment, as measured by the MIRS, and areas of atrophy (236, 296, 243, 295). Labayru et al observed a correlation between increased MIRS and atrophy in primary visual and sensorimotor regions, prefrontal ventromedial and orbitofrontal areas, anterior cingulate cortex, precuneus, left thalamus and bilateral striatum (295); Schneider-Gold et al observed a negative association between MIRS and supratentorial atrophy (243). Zanigni et al (279), using the clinical disability scale for DM (the NIFDS scale), observed that severity of myotonia correlated with a diffuse WM alteration involving also the posterior limb of the internal capsule, the corona radiata adjacent to motor areas and the splenium of corpus callosum; WM differences in the same regions correlated also with the motor domain score of the clinical scale (298). On the contrary, other authors found no correlation between MRI and muscular impairment (277, 237).

Taken together these data reinforce the notion of a central, and not merely muscular, motor dysfunction in adult DM1.

CONCLUSIONS

This is a monocentric and prospective study conducted on a large population of 72 DM1 patients (24 E1, 41 E2 and 7 E3) with the main objective of a wide phenotypic and genotypic characterization of this multisystem disease.

We analyzed the main clinical aspects of this cohort of patients through standardized clinical and instrumental examinations, conducted by a multidisciplinary team of dedicated specialists (neurologists, cardiologists, pneumologist, neuroradiologists, neuropsychologists), belonging to our centre, that followed patients over 3 years of follow-up. We studied the possible correlations among the different clinical and instrumental parameters, neuroimaging data and genotypic aspects, examining the disease in all its different phenotypic aspects.

We found an earlier age at onset and a greater frequency of perinatal problems and delayed psychomotor development in the E3 group of patients, confirming the relationship between CTG expansion size and these clinical parameters in the more severe form of DM1.

We observed a cardiological involvement in about two thirds of patients, with a prevalence of conduction disorders, without significant differences among the 3 genetic classes of expansion. Longer disease duration

was associated with increased PR length, higher incidence of conduction disorders and lower ejection fraction, suggesting a progressive cardiological involvement; we also found that higher CVF values were significant predictors for a reduced risk of cardiopathy, confirming the hypothesis of another study (107).
Patients with conduction disorders had also higher MIRS score. Taken together our results seem to suggest that cardiological involvement is progressive and can be closely associated with the progression of pulmonary and muscle involvement; however, the lack of significant differences among the different genetic classes and the observation that most patients can be asymptomatic confirm the importance of a close cardiological follow-up in these patients.
From a respiratory point of view, in agreement with the literature, we found that respiratory dysfunction, predominantly a restrictive ventilatory pattern, is common in DM1 patients and is associated with alveolar hypoventilation and chronic hypercapnia. In addition, we found a significant correlation between class of expansion and functional pulmonary parameters (CVF, FEV1, MIP and MEP) that showed a significant reduction in E3, compared to E2 and E1 patients; functional pulmonary parameters were also negatively associated with disease duration and MIRS score, confirming the results of some previous studies (122, 138, 150) and with the NIFDS scale. Moreover, we found that higher MIRS values were significant predictors for an increased risk of restrictive syndrome. On the contrary, arterial blood gas parameters (pO2 and pCO2) did not differ among the 3 genetic classes and were not associated with disease duration nor with respiratory function test or MIRS score. However, applying the adjusted logistic regression, higher CVF values were significant predictors for a reduced risk of hypoxemia. Our findings, resulting from a large cohort of DM1 patients, corroborate the hypothesis suggested by other authors that arterial blood gas values are at least in part independent of the grade of muscular impairment, confirming a possible central nervous system (CNS) dysfunction (105, 122, 127, 130). On the other hand, the trend of respiratory function test seems to be significantly associated with clinical muscle severity, even if a recent longitudinal study suggested that decline of respiratory function is not due to progressive muscle impairment alone, depending also from other factors, including the correct use of NIV (138). In order to better interpret our results, a longer follow-up is needed.
Regarding sleep parameters we found a value of pathological daytime sleepiness (EDS) in 16.3% of patients, without significant differences among the 3 genetic classes. In agreement with previous studies (188-190), comparing patients with and without excessive daytime sleepiness, we found no associations with age, disease duration, gender, BMI, MIRS scale, arterial blood gas values, ODI index or AHI index. In addition, we found no relationship with cardiological parameters, while we observed a positive correlation among ESS score, neuropsychological (NP) domain of the NIFDS scale and COMPASS 31 total score and its subitems, suggesting a possible greater disease burden on these patients. The positive association with the NP domain of the NIFDS could suggest that DM1 patients with EDS are more prone to psychological distress and underlines the importance of taking into account also the excessive somnolence when analyzing neuropsychological data.

In agreement with literature, in our study sleep apnoea was detected in a large percentage of DM1 patients, with a predominance of pure obstructive apnoea, even if about one third of patients presented both obstructive/central apnoea and some patients had pure central apnoea. In addition to previous studies, which often lack a genotypic characterization, we found no significant differences between the 3 genetic classes regarding the type and severity of apnoea. Moreover, we observed that higher CVF values were significant predictors for a reduced risk of apnoea and, consistent with this result, we found that ODI index and obstructive AHI and mixed apnoea index had significant negative associations with CVF, FEV1 and diurnal pO2; moreover, both these sleep parameters showed a positive association with the daily life activity (DL) domain of the NIFDS scale, while AHI and mixed apnoea index had also a positive association with PR interval length on ECG. Nocturnal saturation parameters had significant positive correlations with diurnal pulmonary parameters (CVF, FEV1 and pO2) and mean SaO2 showed positive correlation with heart rate and diurnal SaO2. Overall, our results seem to suggest that at least some diurnal parameters could identify patients at lower risk of sleep related breathing disorders (SRBD). In particular, higher CVF values seem to be significant predictors for a reduced risk of apnoea. The positive associations between ODI index and obstructive AHI/mixed apnoea index with NIFDS score, and between obstructive AHI/mixed apnoea index and PR interval length could suggest that patients with more advanced disease and higher disease burden are also at higher risk of SRBD. However, in patients with intermediate levels of disease burden, nocturnal respiratory events seem to be, above all, unpredictable and at least in part independent of the severity of muscular impairment, as suggested by the lack of association with MIRS scale and class of expansion. This observation underlines the importance of studying all DM1 patients with periodic cardiorespiratory monitoring in order to early diagnose SRBD and treat it promptly, avoiding possible cardiovascular and cognitive consequences.

The study of skin biopsy, performed on 45 patients, is one of the most innovative aspects of this study; we demonstrated the presence of a subclinical small fibre neuropathy with a significant involvement of the somatic fibres but not of the autonomic ones. No significant differences were found among the 3 classes of expansion, nor among different groups of age at onset or different groups of MIRS score. We observed that males presented a significantly lower fibre density in the legs compared to females, and that fibre density was lower in the legs compared to the thighs. These preliminary results need to be confirmed by enlarging the number of controls used for the skin biopsy comparison, because the different sample size used could potentially impact on the lack of significance for the autonomic component.

Only some studies conducted on small samples of patients analyzed pupillary function in DM1 patients, with contradictory results. We examined with pupillometry 26 DM1 patients, compared to a group of healthy controls, observing a significantly lower pupil size at baseline and a lower constriction response to light. Lower pupil size at baseline could point to a possible sympathetic dysfunction, and this is further corroborated by the significant correlation with longer disease duration, lower ejection fraction and longer QRS interval length at ECG, overall indicating a greater disease burden. Overall, the sympathetic dysfunction suggested by these results and the correlation with cardiological parameters, should be further explored by evaluating

the autonomic innervation of the heart (using cardiac SPECT MIBG) and, at the same time, the anatomical structural damage of the heart through cardiac MRI. Regarding peak amplitude values, we observed that patients had lower constriction values; moreover, higher constriction response values were associated with higher percentage of autonomic fibres innervating arrector pili muscles in the thigh on skin biopsy, while lower constriction response values were found in patients with higher disability score and higher apnoea/hypopnea index. These results can reflect both parasympathetic dysfunction or possible myotonia of smooth muscle sphincter of the iris, but they must be interpreted with caution since we still have to analyze data on pupillary response dynamics in order to better evaluate the possible myotonic component of the PLR. Finally, 33 patients of our sample (13 E1 and 20 E2) were studied with a full neuropsychological evaluation and a quantitative structural MRI study, and compared to a group of healthy control subjects.

Comparing performance of overall DM1 patients to the normal values, only a small percentage of patients scored lower than cut-offs in MMSE and CPM-47 test, confirming the observation that neuropsychological deficits in DM might partially escape commonly applied neuropsychological test batteries. However, applying more specific tests, we found that the most affected domains were executive functions and visuoconstructional task, attention, visuospatial, visuoperceptual tasks and visuospatial short-term memory, followed by language task (in particular associative fluency task), short-term and long-term verbal memory. Moreover, a discrete percentage of patients performed worse than controls in social cognition test and had scored above cut-offs for the presence of anosognosia. Almost half of patients presented significant levels of apathy and we also found high levels of depression, anxiety and anger. Overall, our results confirmed, on a large cohort of DM1 patients, the observation of previous studies (147, 237, 240-243, 336, 251, 252, 344-349). A high percentage of DM1 patients reported a worsened quality of life and about half of patients reported a significant level of physical pain and worsened ratio of energy/fatigue in daily activities. In addition to previous studies, comparing E1 and E2 patients, we found a worse performance of E1 patients in the visuoperceptual ability and social cognition tasks, while E2 patients showed a significantly higher global score of apathy and a significant index of mild trait anxiety, suggesting a higher predisposition to react anxiously to a situation. We also observed that all cognitive reserve indices were significantly higher in E1 compared to E2 patients, showing the presence of a higher number of protective factors, such as educational level, occupation and leisure time activities, enhancing the ability to optimize and maximize cognitive performance. E1 patients reported a generally higher level of quality of life in all investigated domains, in particular in the domains of physical health, perceived limits due to emotional problems and emotional well-being. Regarding MRI morphological study, we observed an enlargement of the lateral ventricular volumes and a decrease of volumes in frontal, parietal, temporal and occipital cortices; at the subcortical level, we found a bilateral decreased volume of accumbens and putamen nuclei, confirming results of previous studies (283, 286). In addition, comparing the 2 subgroups of DM1 patients, we found a more severe volume reduction of the isthmus cingulate, the transverse temporal, the superior parietal and temporal gyri in E2 patients. On the contrary, we did not find significant differences in white matter lesion load between the 2 genetic classes of patients.

As concerns correlations between MRI data and neuropsychological assessment, we observed a negative association between left lateral ventricle enlargement and performance on short-term verbal memory task, while both left lateral and third ventricle's enlargement negatively correlated with emotion attribution abilities in a social cognition task. At the cortical level, the volume of right superior temporal sulcus banks positively correlated with nonverbal reasoning scores. Positive correlations were also found between left lateral occipital volume and performance on visuoperceptive and long-term visuospatial memory tasks.

Right middle temporal volume was positively correlated with both visuoconstructional and visuoperceptive abilities, while it negatively correlated with both total score and autonomy in daily activities subitems' scores on the ICS questionnaire. Finally, right superior temporal volume was negatively correlated with executive function abilities.

In addition, in our study we analyzed possible associations between MRI data and the other clinical data collected, in particular with pulmonary function, sleep parameters, severity of muscular involvement and cardiological parameters, in order to assess a possible CNS involvement in regulating these functions.

We observed that left middle temporal volume was positively correlated with respiratory parameters (diurnal pO2 and nocturnal mean SaO2), while a negative correlation was found between left supramarginal volume and central apnoea index. At a subcortical level we found a negative correlation between left and right putamen nuclei volume reduction and O2 saturation levels, and a positive correlation between volume reduction of left putamen nucleus and diurnal pO2. To the best of our knowledge only some authors have analyzed the possible relationships between neuroimaging and sleep apnoea in DM1 (285), while no study has analyzed the association between diurnal pulmonary parameters and MRI data.

In conclusion, the main strength of our study, compared to previous literature, is the fact that we analyzed a wide spectrum of clinical parameters in a large cohort of DM1 patients, in a monocentric and prospective study, which allowed a standardized clinical and instrumental evaluation of these patients that were followed up over time by dedicated specialists in a multidisciplinary team.

We examined all the main clinical aspects of the pathology, looking for possible correlations between the different clinical and instrumental parameters, genotypic characteristics and neuroimaging findings.

Our finding corroborates results emerging from previous studies, which nevertheless were conducted on small populations of patients, sometimes were retrospective or multicentric without the possibility of applying standardized protocols or lacking genotypic characterization. Moreover, most of the previous studies analyzed single clinical aspects separately, while we have taken into account the wide phenotypic spectrum of the pathology, correlating parameters belonging to different clinical axes to each other, with the genetic aspects and neuroimaging findings. In order to better understand the pathophysiology of DM1 clinical aspects and treat it efficiently, it is fundamental that we study the interplay between neurological, respiratory and cardiological involvement, as suggested by the observation that sleep disorders can affect mood and cognition and sleep breathing disorders can result in cardiovascular and pulmonary morbidity.

From our results what emerged was that the variability of the multisystem aspects of this pathology is, at least in part, independent of the genotype and, therefore, difficult to predict, underlying the importance of a multidisciplinary periodic follow-up.

The natural history of many aspects of DM1 pathology is largely unclear, thus systematic longitudinal studies applying standardized clinical, neuropsychological and brain imaging protocols on large cohorts of genetically and clinically well characterized participants are needed.

We therefore believe that our study contributed significantly to the phenotypic and genotypic characterization of this pathology and could constitute the basis of a more prolonged follow-up of these patients, with the objective of enlarging the collection of biological samples and performing more in-depth instrumental analyses aimed at clarifying the most difficult to interpret results we have obtained.

REFERENCES

1. Sansone VA. The Dystrophic and Non dystrophic Myotonias. Continuum (Minneap Minn). 2016 Dec;22(6, Muscle and Neuromuscular Junction Disorders):1889-1915.
2. Lossin C, George AL Jr. Myotonia congenita. Adv Genet. 2008;63:25-55.
3. Wong E. Periodic paralysis and related disorders- Medlink Neurology
4. Meola G, Cardani R. Myotonic dystrophies: An update on clinical aspects, genetic, pathology, and molecular pathomechanisms. Biochim Biophys Acta. 2015 Apr;1852(4):594-606.
5. 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) 172:572–80.
6. Udd B, Krahe R: The myotonic dystrophies: molecular, clinical, and therapeutic challenges. Lancet Neurol 2012;11:891–905.
7. Wenninger S, Montagnese F, Schoser B. Core Clinical Phenotypes in Myotonic Dystrophies. Front Neurol. 2018 May 2;9:303.
8. Stokes M, Varughese N, Iannaccone S, Castro D.Clinical and genetic characteristics of childhood-onset myotonic dystrophy. Mathew Muscle Nerve. 2019 Dec;60(6):732-738.
9. Wenninger S, Montagnese F, Schoser B. Core clinical phenotypes in myotonic dystrophies. Front Neurol. 2018;9:303.
10. Brunner HG, Bruggenwirth HT, Nillesen W, Jansen G, Hamel BC, Hoppe RL, et al. Influence of sex of the transmitting parent as well as of parental allele size on the CTG expansion in myotonic dystrophy (DM). Am J Hum Genet(1993) 53(5):1016–23.
11. Harper PS, Dyken PR. Early-onset dystrophia myotonica. Evidence supporting a maternal environmental factor. Lancet (1972) 2(7767):53–5.
12. Zeesman S, Carson N, Whelan DT. Paternal transmission of the congenital form of myotonic dystrophy type 1: a new case and review of the literature. Am J Med Genet (2002) 107(3):222–6.
13. Angeard N, Gargiulo M, Jacquette A, Radvanyi H, Eymard B, Heron D. Cognitive profile in childhood myotonic dystrophy type 1: is there a global impairment? Neuromuscul Disord (2007) 17(6):451–8.
14. Wallgren-Pettersson C, Bushby K, Mellies U, Simonds A. 117th ENMC workshop: ventilatory support in congenital neuromuscular disorders – congenital myopathies, congenital muscular dystrophies, congenital myotonic dystrophy and SMA (II) 4-6 April 2003, Naarden, The Netherlands. Neuromuscul Disord (2004) 14(1):56–69.
15. Mathieu J, Allard P, Potvin L, Prevost C, Begin P. A 10-year study of mortality in a cohort of patients with myotonic dystrophy. Neurology (1999) 52(8):1658–62.
16. Ekstrom AB, Hakenas-Plate L, Samuelsson L, Tulinius M, Wentz E. Autism spectrum conditions in myotonic dystrophy type 1: a study on 57 individuals with congenital and childhood forms. Am J Med Genet B Neuropsychiatr Genet (2008) 147B(6):918–26.
17. Douniol M, Jacquette A, Cohen D, Bodeau N, Rachidi L, Angeard N, et al. Psychiatric and cognitive phe- notype of childhood myotonic dystrophy type 1. Dev Med Child Neurol. (2012) 54:905–11.
18. Douniol M, Jacquette A et al. Psychiatric and cognitive phenotype in children and adolescents with myotonic dystrophy Eur Child Adolesc Psychiatry. 2009 Dec;18(12):705-15
19. Ho G, Cardamone M, Farrar M. Congenital and childhood myotonic dystrophy: current aspects of disease and future directions. World J Clin Pediatr(2015) 4(4):66–80.
20. Meola G. Myotonic dystrophy type 2: the 2020 update. Acta Myol. 2020 Dec 1;39(4):222-234.
21. Day JW, Ricker K, Jacobsen JF, et al. Myotonic dystrophy type 2: molecular, diagnostic and clinical spectrum. Neurology 2003;60:657-64.
22. Sansone VA, Brigonzi E, Schoser B, Villani S, Gaeta M, De Ambroggi G et al (2013) The frequency and severity of cardiac involvement in myotonic dystrophy type 2 (DM2): long-term outcomes. Int J Cardiol 168(2):1147–1153
23. Minnerop M, Weber B, Schoene-Bake JC, Roeske S, Mirbach S, Anspach C, et al. The brain in myotonic dystrophy 1 and 2: evidence for a predominant white matter disease. Brain (2011) 134(Pt 12):3530–46. doi: 10.1093/brain/awr299
24. C.A. Thornton, R.C. Griggs, R.T. Moxley III, Myotonic dystrophy with no trinucleotide repeat expansion, Ann. Neurol. 35 (1994) 269–272.
25. H.G. Brunner, H.T. Bruggenwirth, W. Nillesen, G. Jansen, B.C. Hamel, R.L. Hoppe, C.E. de Die, C.J. Höweler, B.A. van Oost, B. Wieringa, H.H. Ropers, H.J.M. Smeets, Influence of sex of the transmitting parent as well as of parental allele size on the CTG expansion in myotonic dystrophy (DM), Am. J. Hum. Genet. 53 (1993) 1016–1023.
26. C.E. de Die-Smulders, H.J. Smeets, W. Loots, H.B. Anten, J.F. Mirandolle, J.P. Geraedts, C.J. Höweler, Paternal transmission of congenital myotonic dystrophy, J. Med. Genet. 34 (1997) 930–933.
27. T. Ashizawa, M. Anvret, M. Baiget, J.M. Barcelo, H. Brunner, A.M. Cobo, B. Dallapiccola, R.G. Fenwick Jr., U. Grandell, H. Harley, C. Junien, M.C. Koch, R.G. Korneluk, C. Lavedan, T. Miki, J.C. Mulley, A. Lopez de Munain, G. Novelli, A.D. Roses, W.K. Seltzer, D.J. Shaw, H. Smeets, G.R. Sutherland, H. Yamagata, P.S. Harper, Characteristics of intergenerational contractions of the CTG repeat in myotonic dystrophy, Am. J. Hum. Genet. 54 (1994) 414–423.

28. J. Puymirat, Y. Giguère, J. Mathieu, J.P. Bouchard, Intergenerational contraction of CTG repeats in 2 families with myotonic dystrophy type 1, Neurology 73 (2009) 2126–2127.
29. Ballester-Lopez A, Koehorst E at al Preliminary Findings on CTG Expansion Determination in Different Tissues from Patients with Myotonic Dystrophy Type 1 Genes (Basel). 2020 Nov 7;11(11):1321
30. E.P. Leeflang, N. Arnheim, A novel repeat structure at the myotonic dystrophy locus in a 37 repeat allele with unexpectedly high stability, Hum. Mol. Genet. 4 (1995) 135–136.
31. Z. Musova, R. Mazanec, A. Krepelova, E. Ehler, J. Vales, R. Jaklova, T. Prochazka, P. Koukal, T. Marikova, J. Kraus, M. Havlovicova, Z. Sedlace, Highly unstable sequence interruptions of the CTG repeat in the myotonic dystrophy gene, Am. J. Med. Genet. A 149A (2009) 1365–1374.
32. C. Braida, R.K. Stefanatos, B. Adam, N. Mahajan, H.J. Smeets, F. Niel, C. Goizet, B. Arveiler, M. Koenig, C. Lagier-Tourenne, J.L. Mandel, C.G. Faber, C.E. de DieSmulders, F. Spaans, D.G. Monckton, Variant CCG and GGC repeats within the CTG expansion dramatically modify mutational dynamics and likely contribute toward unusual symptoms in some myotonic dystrophy type 1 patients, Hum. Mol. Genet. 19 (2010) 1399–1412.
33. M. Santoro, M. Masciullo, R. Pietrobono, G. Conte, A. Modoni, M.L.E. Bianchi, V. Rizzo, M.G. Pomponi, G. Tasca, G. Neri, G. Silvestri, Molecular, clinical, and muscle studies in myotonic dystrophy type 1 (DM1) associated with novel variant CCG expansions, J. Neurol. 260 (2013) 1245–1257
34. Liquori CL, Ricker K, Moseley ML, Jacobsen JF, Kress W, Naylor SL, et al. Myotonic dystrophy type 2 caused by a CCTG expansion in intron 1 of ZNF9. Science. 2001;293:864–7.
35. L.L. Bachinski, B. Udd, G. Meola, V. Sansone, G. Bassez, B. Eymard, C.A. Thornton, R.T. Moxley, P.S. Harper, M.T. Rogers, K. Jurkat-Rott, F. Lehmann-Horn, T. Wieser, J. Gamez, C. Navarro, A. Bottani, A. Kohler, M.D. Shriver, R. Sallinen, M. Wessman, S. Zhang, F.A. Wright, R. Krahe, Confirmation of the type 2 myotonic dystrophy (CCTG)n expansion mutation in patients with proximal myotonic myopathy/proximal myotonic dystrophy of different European origins: a single shared haplotype indicates an ancestral founder effect, Am. J. Hum. Genet. 73 (2003) 835–848.
36. L.L. Bachinski, T. Czernuszewicz, L.S. Ramagli, T. Suominen, M.D. Shriver, B. Udd, M. J. Siciliano, R. Krahe, Premutation allele pool in myotonic dystrophy type 2, Neurology 72 (2009) 490–497.
37. J.W. Day, K. Ricker, J.F. Jacobsen, L.J. Rasmussen, K.A. Dick, W.C. Kress, M.C. Koch, G. J. Beilman, A.R. Harrison, J.C. Dalton, L.P. Ranum, Myotonic dystrophy type 2: molecular, diagnostic and clinical spectrum, Neurology 60 (2003) 657–664.
38. L.J. Wong, T. Ashizawa, D.G. Monckton, C.T. Caskey, C.S. Richards, Somatic heterogeneity of the CTG repeat in myotonic dystrophy is age and size dependent, Am. J. Hum. Genet. 56 (1995) 114–122.
39. D.G. Monckton, L.I. Wong, T. Ashizawa, C.T. Caskey, Somatic mosaicism, germline expansions, germline reversions and intergenerational reductions in myotonic dystrophy males: small pool PCR analyses, Hum. Mol. Genet. 4 (1995) 1–8.
40. T. Suominen, B. Schoser, O. Raheem, S. Auvinen, M. Walter, R. Krahe, H. Lochmuller, W. Kress, B. Udd, High frequency of co-segregating CLCN1 mutations among myotonic dystrophy type 2 patients from Finland and Germany, J. Neurol. 255 (2008) 1731–1736.
41. R. Cardani, M. Giagnacovo, A. Botta, F. Rinaldi, A. Morgante, B. Udd, O. Raheem, S. Penttila, T. Suominen, L.V. Renna, V. Sansone, E. Bugiardini, G. Novelli, G. Meola. Co-segregation of DM2 with a recessive CLCN1 mutation in juvenile onset of myotonic dystrophy type 2, J. Neurol. 259 (2012) 2090–2099.
42. Bugiardini E, Rivolta I, Binda A, Soriano Caminero A, Cirillo F, Cinti A, et al. SCN4A mutation as modifying factor of myotonic dystrophy type 2 phenotype. Neuromuscul Disord (2015) 25(4):301–7.
43. A.V. Philips, L.T. Timchenko, T.A. Cooper, Disruption of splicing regulated by a CUGbinding protein in myotonic dystrophy, Science 280 (1998) 737–741.
44. J.W. Miller, C.R. Urbinati, P. Teng-Umnuay, M.G. Stenberg, B.J. Byrne, C.A. Thornton, M.S. Swanson, Recruitment of human muscleblind proteins to (CUG) (n) expansions associated with myotonic dystrophy, EMBO J. 19 (2000) 4439–4448.
45. R.N. Kanadia, K.A. Johnstone, A. Mankodi, C. Lungu, C.A. Thornton, D. Esson, A.M. Timmers, W.W. Hauswirth, M.S. Swanson, A muscleblind knockout model for myotonic dystrophy, Science 302 (2003) 1978–1980.
46. T.H. Ho, N. Charlet-B, M.G. Poulos, G. Singh, M.S. Swanson, T.A. Cooper, Muscleblind proteins regulate alternative splicing, EMBO J. 23 (2004) 3103–3112.
47. S. Paul, W. Dansithong, D. Kim, J. Rossi, N.J. Webster, L. Comai, S. Reddy, Interaction of muscleblind, CUG-BP1 and hnRNP H proteins in DM1-associated aberrant IR splicing, EMBO J. 25 (2006) 4271–4283.
48. A. Ravel-Chapuis, G. Belanger, R.S. Yadava, M.S. Mahadevan, L. DesGroseillers, J. Cote, B.J. Jasmin, The RNA-binding protein Staufen1 is increased in DM1 skeletal muscle and promotes alternative pre-mRNA splicing, J. Cell Biol. 196 (2012)
49. I. Holt, V. Jacquemin, M. Fardaei, C.A. Sewry, G.S. Butler-Browne, D. Furling, J.D. Brook, G.E. Morris, Muscleblind-like proteins: similarities and differences in normal and myotonic dystrophy muscle, Am. J. Pathol. 174 (2009) 216–227.
50. K. Charizanis, K.Y. Lee, R. Batra, M. Goodwin, C. Zhang, Y. Yuan, L. Shiue, M. Cline, M. M. Scotti, G. Xia, A. Kumar, T. Ashizawa, H.B. Clark, T. Kimura, M.P. Takahashi, H. Fujimura, K. Jinnai, H. Yoshikawa, M. Gomes-Pereira, G.

Gourdon, N. Sakai, S. Nishino, T.C. Foster, M. Ares Jr., R.B. Darnell, M.S. Swanson, Muscleblind-like 2- mediated alternative splicing in the developing brain and dysregulation in myotonic dystrophy, Neuron 75 (2012) 437–450.
51. E.T. Wang, N.A. Cody, S. Jog, M. Biancolella, T.T. Wang, D.J. Treacy, S. Luo, G.P. Schroth, D.E. Housman, S. Reddy, E. Lécuyer, C.B. Burge, Transcriptome-wide regulation of premRNA splicing and mRNA localization by muscleblind proteins, Cell 150 (2012) 710–724.
52. C. Huichalaf, K. Sakai, B. Jin, K. Jones, G.L. Wang, B. Schoser, C. Schneider-Gold, P. Sarkar, O.M. Pereira-Smith, N. Timchenko, L. Timchenko, Expansion of CUG RNA repeats causes stress and inhibition of translation in myotonic dystrophy 1(DM1) cells, FASEB J. 24 (2010) 3706–3719.
53. W. Dansithong, S. Paul, L. Comai, S. Reddy, MBNL1 is the primary determinant of focus formation and aberrant insulin receptor splicing in DM1, J. Biol. Chem. 280 (2005) 5773–5780.
54. N.A. Timchenko, G.L. Wang, L.T. Timchenko, RNA CUG-binding protein 1 increases translation of 20-kDa isoform of CCAAT/enhancer-binding protein beta by interacting with the alpha and beta subunits of eukaryotic initiationtranslation factor 2, J. Biol. Chem. 280 (2005) 20549–20557.
55. C. Barreau, L. Paillard, A. Mereau, H.B. Osborne, Mammalian CELF/Bruno-like RNAbinding proteins: molecular characteristics and biological functions, Biochimie 88 (2006) 515–525.
56. C. Huichalaf, B. Schoser, C. Schneider-Gold, B. Jin, P. Sarkar, L. Timchenko, Reduction of the rate of protein translation in patients with myotonic dystrophy 2, J. Neurosci. 29 (2009) 9042–9049.
57. E. Salisbury, K. Sakai, B. Schoser, C. Huichalaf, C. Schneider-Gold, H. Nguyen, G.L. Wang, J.H. Albrecht, L.T. Timchenko, Ectopic expression of cyclin D3 corrects differentiation of DM1 myoblasts through activation of RNA CUG-binding protein, CUGBP1, Exp. Cell Res. 314 (2008) 2266–2278.
58. López-Martínez A, Soblechero-Martín P, de-la-Puente-Ovejero L, Nogales-Gadea G, Arechavala-Gomeza V. An Overview of Alternative Splicing Defects Implicated in Myotonic Dystrophy Type I . Genes 2020, 11, 1109.
59. Jiang, H.; Mankodi, A.; Swanson, M.S.; Moxley, R.T.; Thornton, C.A. Myotonic dystrophy type 1 is associated with nuclear foci of mutant RNA, sequestration of muscleblind proteins and deregulated alternative splicing in neurons. Hum. Mol. Genet. 2004, 13, 3079–3088.
60. Goodwin, M.; Mohan, A.; Batra, R.; Lee, K.Y.; Charizanis, K.; Fernández Gómez, F.J.; Eddarkaoui, S.; Sergeant, N.; Buée, L.; Kimura, T.; et al. MBNL Sequestration by Toxic RNAs and RNA Misprocessing in the Myotonic Dystrophy Brain. Cell Rep. 2015.
61. Yamashita, Y.; Matsuura, T.; Shinmi, J.; Amakusa, Y.; Masuda, A.; Ito, M.; Kinoshita, M.; Furuya, H.; Abe, K.; Ibi, T.; et al. Four parameters increase the sensitivity and specificity of the exon array analysis and disclose novel aberrantly spliced exons in myotonic dystrophy. J. Hum. Genet. 2012, 57, 368–374.
62. Dhaenens, C.M.; Schraen-Maschke, S.; Tran, H.; Vingtdeux, V.; Ghanem, D.; Leroy, O.; Delplanque, J.; Vanbrussel, E.; Delacourte, A.; Vermersch, P.; et al. Overexpression of MBNL1 fetal isoforms and modified splicing of Tau in the DM1 brain: Two individual consequences of CUG trinucleotide repeats. Exp. Neurol. 2008, 210, 467–478
63. Gao, Z.; Cooper, T.A. Reexpression of pyruvate kinase M2 in type 1 myofibers correlates with altered glucose metabolism in myotonic dystrophy. Proc. Natl. Acad. Sci. USA 2013, 110, 13570–13575.
64. Charlet, B.N.; Savkur, R.S.; Singh, G.; Philips, A.V.; Grice, E.A.; Cooper, T.A. Loss of the muscle-specific chloride channel in type 1 myotonic dystrophy due to misregulated alternative splicing. Mol. Cell 2002, 10, 45–53
65. Mankodi, A.; Takahashi, M.P.; Jiang, H.; Beck, C.L.; Bowers, W.J.; Moxley, R.T.; Cannon, S.C. Thornton, C.A. Expanded CUG Repeats Trigger Aberrant Splicing of ClC-1 Chloride Channel Pre-mRNA and Hyperexcitability of Skeletal Muscle in Myotonic Dystrophy. Mol. Cell 2002, 10, 35–44.
66. Nakamori, M.; Sobczak, K.; Puwanant, A.; Welle, S.; Eichinger, K.; Pandya, S.; Dekdebrun, J.; Heatwole, C.R.; McDermott, M.P.; Chen, T.; et al. Splicing biomarkers of disease severity in myotonic dystrophy. Ann. Neurol. 2013, 74, 862–872.
67. Tang, Z.Z.; Yarotskyy, V.; Wei, L.; Sobczak, K.; Nakamori, M.; Eichinger, K.; Moxley, R.T.; Dirksen, R.T.; Thornton, C.A. Muscle weakness in myotonic dystrophy associated with misregulated splicing and altered gating of Ca(V)1.1 calcium channel. Hum. Mol. Genet. 2012, 21, 1312–1324.
68. Fugier, C.; Klein, A.F.; Hammer, C.; Vassilopoulos, S.; Ivarsson, Y.; Toussaint, A.; Tosch, V.; Vignaud, A.; Ferry, A.; Messaddeq, N.; et al. Misregulated alternative splicing of BIN1 is associated with T tubule alterations and muscle weakness in myotonic dystrophy. Nat. Med. 2011, 17, 720–725.
69. Kimura, T.; Nakamori, M.; Lueck, J.D.; Pouliquin, P.; Aoike, F.; Fujimura, H.; Dirksen, R.T.; Takahashi, M.P. Dulhunty, A.F.; Sakoda, S. Altered mRNA splicing of the skeletal muscle ryanodine receptor and sarcoplasmic/endoplasmic reticulum Ca2+-ATPase in myotonic dystrophy type 1. Hum. Mol. Genet. 2005, 14, 2189–2200.
70. Kimura, T.; Nakamori, M.; Lueck, J.D.; Pouliquin, P.; Aoike, F.; Fujimura, H.; Dirksen, R.T.; Takahashi, M.P.; Dulhunty, A.F.; Sakoda, S. Altered mRNA splicing of the skeletal muscle ryanodine receptor and sarcoplasmic/endoplasmic reticulum Ca2+-ATPase in myotonic dystrophy type 1. Hum. Mol. Genet. 2005, 14, 2189–2200

71. Yamashita, Y.; Matsuura, T.; Shinmi, J.; Amakusa, Y.; Masuda, A.; Ito, M.; Kinoshita, M.; Furuya, H.; Abe, K.; Ibi, T.; et al. Four parameters increase the sensitivity and specificity of the exon array analysis and disclose 25 novel aberrantly spliced exons in myotonic dystrophy. J. Hum. Genet. 2012, 57, 368–374
72. Rau, F.; Laine, J.; Ramanoudjame, L.; Ferry, A.; Arandel, L.; Delalande, O.; Jollet, A.; Dingli, F.; Lee, K.Y.; Peccate, C.; et al. Abnormal splicing switch of DMD's penultimate exon compromises muscle fibre maintenance in myotonic dystrophy. Nat. Commun. 2015, 6, 7205
73. Koebis, M.; Ohsawa, N.; Kino, Y.; Sasagawa, N.; Nishino, I.; Ishiura, S. Alternative splicing of myomesin 1 gene is aberrantly regulated in myotonic dystrophy type 1. Genes Cells 2011, 16, 961–972. [
74. Savkur, R.S.; Philips, A.V.; Cooper, T.A. Aberrant regulation of insulin receptor alternative splicing is associated with insulin resistance in myotonic dystrophy. Nat. Genet. 2001, 29, 40–47
75. Freyermuth, F.; Rau, F.; Kokunai, Y.; Linke, T.; Sellier, C.; Nakamori, M.; Kino, Y.; Arandel, L.; Jollet, A.; Thibault, C.; et al. Splicing misregulation of SCN5A contributes to cardiac-conduction delay and heart arrhythmia in myotonic dystrophy. Nat. Commun. 2016, 7, 11067.
76. Dixon, D.M.; Choi, J.; El-Ghazali, A.; Park, S.Y.; Roos, K.P.; Jordan, M.C.; Fishbein, M.C.; Comai, L.; Reddy, S. Loss of muscleblind-like 1 results in cardiac pathology and persistence of embryonic splice isoforms. Sci. Rep. 2015, 5, 9042.
77. T. Zu, B. Gibbensa, N.S. Dotya, M. Gomes-Pereira, A. Huguetd, M.D. Stone, J. Margolis, M. Petersong, T.W. Markowski, M.A.C. Ingram, Z. Nan, C. Forster, W.C. Low, B. Schoser, N.V. Somia, H.B. Clark, S. Schmechel, P.B. Bitterman, G. Gourdon, M.S. Swanson, M. Moseley, L.P.W. Ranum, Non-ATG-initiated translation directed by microsatellite expansions, Proc. Natl. Acad. Sci. U. S. A. 108 (2011) 260–265.
78. I. Eisenberg, M.S. Alexander, L.M. Kunkel, miRNAS in normal and diseased skeletal muscle, J. Cell. Mol. Med. 13 (2009) 2–11.
79. S. Greco, M. De Simone, C. Colussi, G. Zaccagnini, P. Fasanaro, M. Pescatori, R. Cardani, R. Perbellini, E. Isaia, P. Sale, G. Meola, M.C. Capogrossi, C. Gaetano, F. Martelli, Common micro-RNA signature in skeletal muscle damage and regeneration induced by Duchenne muscular dystrophy and acute ischemia, FASEB J. 23 (2009) 3335–3346.
80. S. Gambardella, F. Rinaldi, S.M. Lepore, A. Vihola, E. Loro, C. Angelini, L. Vergani, G. Novelli, A. Botta, Overexpression of microRNA-206 in the skeletal muscle from myotonic dystrophy type 1 patients, J. Transl. Med. 8 (2010) 48–54.
81. R. Perbellini, S. Greco, G. Sarra-Ferraris, R. Cardani, M.C. Capogrossi, G. Meola, F. Martelli, Dysregulation and cellular mislocalization of specific miRNAs in myotonic dystrophy type 1, Neuromuscul. Disord. 21 (2011) 81–88.
82. S. Greco, A. Perfetti, P. Fasanaro, R. Cardani, M.C. Capogrossi, G. Meola, F. Martelli, Deregulated microRNAs in myotonic dystrophy type 2, PLoS One 7 (2012) e39732.
83. F. Rau, F. Freyermuth, C. Fugier, J.P. Villemin, M.C. Fischer, B. Jost, D. Dembele, G. Gourdon, A. Nicole, D. Duboc, K. Wahbi, J.W. Day, H. Fujimura, M.P. Takahashi, D. Auboeuf, N. Dreumont, D. Furling, N. Charlet-Berguerand, Misregulation of miR-1 processing is associated with heart defects in myotonic dystrophy, Nat. Struct. Mol. Biol. 18 (2011) 840–845.
84. Perfetti A, Greco S, Bugiardini E, et al. Plasma microRNAs as biomarkers for myotonic dystrophy type 1. Neuromuscular Disord 2014;24(6):509-15. PMID 24679513
85. Perfetti A, Greco S, Cardani R, et al. Validation of plasma microRNAs as biomarkers for myotonic dystrophy type 1. Sci Rep 2016;6:38174. PMID 27905532
86. Itoh H, Hisamatsu T, Cardiac Conduction Disorders as Markers of Cardiac Events in Myotonic Dystrophy Type 1. J Am Heart Assoc. 2020 Sep;9(17):e015709.
87. Groh WJ, Groh MR, Saha C, Kincaid JC, Simmons Z, Ciafaloni E, Pourmand R, Otten RF, Bhakta D, Nair GV, Marashdeh MM, Zipes DP, Pascuzzi RM Electrocardiographic abnormalities and sudden death in myotonic dystrophy type 1. N Engl J Med. 2008;358:2688–2697.
88. Elizabeth M McNally, Douglas L Mann et al. Clinical Care Recommendations for Cardiologists Treating Adults With Myotonic Dystrophy. J Am Heart Assoc. 2020 Feb 18;9(4):e014006.
89. Meola G, Cardani R (2015) Myotonic dystrophies: an update on clinical aspects, genetic, pathology, and molecular pathomechanisms. Biochem Biophys Acta 1852:594–606
90. Wahbi K, Meune C, Bécane HM, Laforêt P, Bassez G, Lazarus A et al (2009) Left ventricular dysfunction and cardiac arrhythmias are frequent in type 2 myotonic dystrophy: a case control study. Neuromuscul Disord 19(7):468–47
91. Choudhary P, Nandakumar R, Greig H, Broadhurst P, Dean J, Puranik R et al (2016) Structural and electrical cardiac abnormalities are prevalent in asymptomatic adults with myotonic dystrophy. Heart 102(18):1472–1478
92. Schara U, Schoser BGH (2006) Myotonic dystrophies type 1 and 2: a summary on current aspects. Semin Pediatr Neurol 13(2):71–79
93. Stojan Peric, Bogdan Bjelica, Ksenija Aleksic, Masa Kovacevic, Edita Cvitan, Gorana Mandic Stojmenovic, Vidosava Rakocevic Stojanovic. Heart involvement in patients with myotonic dystrophy type 2. Acta Neurol Belg 2019 Mar;119(1):77-82. doi: 10.1007/s13760-018-1052-3. Epub 2018 Dec 7.
94. Dogan C, De Antonio M, Hamroun D et al (2016) Gender as a modifying factor infuencing myotonic dystrophy type 1 phenotype severity and mortality: a nationwide multiple databases cross-sectional observational study. PLoS ONE 11:e0148264.

95. Petri H, Vissing J, Witting N et al (2012) Cardiac manifestations of myotonic dystrophy type 1. Int J Cardiol 160:82–88.
96. W. Groh, M. Lowe, D. Zipes, Severity of cardiac conduction involvement and arrhythmias in myotonic dystrophy type 1 correlates with age and CTG repeat length, J. Cardiovasc. Electrophysiol. 13 (5) (2002) 444–448.
97. J.K. Lau R.W. Sy, A. Corbett, L. Kritharideset al Myotonic dystrophy and the heart: A systematic review of evaluation and management. International Journal of Cardiology 184 (2015) 600–608 6.
98. P. Cudia, P. Bernasconi, R. Chiodelli, F. Mangiola, F. Bellocci, A. Dello Russo, et al., Risk of arrhythmia in type I myotonic dystrophy: the role of clinical and genetic variables, J. Neurol. Neurosurg. Psychiatry 80 (7) (2009 Jul) 790–793.
99. Bucci E, Testa M, Licchelli L et al (2018) A 34-year longitudinal study on long-term cardiac outcomes in DM1 patients with normal ECG at baseline at an Italian clinical centre. J Neurol 265:885–895. H
100. Y. Hayashi, U. Ikeda, T. Kojo, M. Nishinaga, H. Miyashita, T. Kuroda, et al., Cardiac abnormalities and cytosine–thymine–guanine trinucleotide repeats in myotonic dystrophy, Am. Heart J. 134 (2 Pt 1) (1997 Aug) 292–297.
101. P. Melacini, C. Villanova, E. Menegazzo, G. Novelli, G. Danieli, G. Rizzoli, et al., Correlation between cardiac involvement and CTG trinucleotide repeat length in myotonic dystrophy, JACC 25 (1) (1995) 239–245.
102. B.A. Hardin, M.R. Lowe, D. Bhakta, W.J. Groh, Heart rate variability declines with increasing age and CTG repeat length in patients with myotonic dystrophy type 1 Ann. Noninvasive Electrocardiol. 8 (3) (2003 Jul) 227–232.
103. N.R. Clarke, A.D. Kelion, J. Nixon, D. Hilton-Jones, J.C. Forfar, Does cytosine–thymine–guanine (CTG) expansion size predict cardiac events and electrocardiographic progression in myotonic dystrophy? Heart 86 (4) (2001 Oct) 411–416
104. D. Bhakta, M.R. Lowe, W.J. Groh, Prevalence of structural cardiac abnormalities in patients with myotonic dystrophy type I, Am. Heart J. 147 (2) (2004 Feb)224–227.
105. A.M. Hawkins, C.L. Hawkins, K. Abdul RazakA,, T.K. Khooa, K. Trana, R.V. Jacksona, Respiratory dysfunction in myotonic dystrophy type 1: A systematic review. Neuromuscul Disord. 2019 Mar;29(3):198-212.
106. Salvatore Rossi, Giacomo Della Marca, Martina Ricci, Alessia Perna, Tommaso F Nicoletti, Valerio Brunetti, Emiliana Meleo, Mariarosaria Calvello, Antonio Petrucci, Giovanni Antonini, Elisabetta Bucci, Loretta Licchelli, Cristina Sancricca, Roberto Massa, Emanuele Rastelli, Annalisa Botta, Antonio Di Muzio, Sonia Romano, Matteo Garibaldi, Gabriella Silvestri. Prevalence and predictor factors of respiratory impairment in a large cohort of patients with Myotonic Dystrophy type 1 (DM1): A retrospective, cross sectional study. J Neurol Sci 2019 Apr 15;399:118-124.
107.. Kaminsky P, Brembilla-Perrot B, Pruna L, Poussel M, Chenuel B. Age, conduction defects and restrictive lung disease independently predict cardiac events and death in myotonic dystrophy. Int J Cardiol 2013;162(3):172–8.
108. C. Marchini, R. Lonigro, L. Verriello, L. Pellizzari, P. Bergonzi, G. Damante, Correlations between individual clinical manifestations and CTG repeat amplification in myotonic dystrophy, Clin. Genet. 57 (1) (2000 Jan) 74–82.
109. M. Parisi, M. Galderisi, M. Sidiropulos, C. Fiorillo, R. Lanzillo, A. D'Errico, et al., Early detection of biventricular involvement in myotonic dystrophy by tissue Doppler,Int. J. Cardiol. 118 (2) (2007 May 31) 227–232.
110. R. Breton, J. Mathieu, Usefulness of clinical and electrocardiographic data for predicting adverse cardiac events in patients with myotonic dystrophy, Can. J. Cardiol. 25 (2) (2009) e23–e27.
111. M.C. Hermans, C.G. Faber, S.C. Bekkers, C.E. de Die-Smulders, M.M. Gerrits, I.S. Merkies, et al., Structural and functional cardiac changes in myotonic dystrophy type 1: a cardiovascular magnetic resonance study, J. Cardiovasc. Magn. Reson. 14(2012) 48.
112. K. Merlevede, D. Vermander, P. Theys, E. Legius, H. Ector, W. Robberecht, Cardiac involvement and CTG expansion in myotonic dystrophy, J. Neurol. 249 (6) (2002 Jun) 693–698.
113. M. Šabovič, I. Medica, N. Logar, E. Mandić, J. Zidar, B. Peterlin, Relation of CTG expansion and clinical variables to electrocardiogram conduction abnormalities and sudden death in patients with myotonic dystrophy, Neuromuscul. Disord. 13 (10) (2003) 822–826.
114. C.A. Thornton, K. Johnson, R.T. Moxley 3rd., Myotonic dystrophy patients have larger CTG expansions in skeletal muscle than in leukocytes, Ann. Neurol. 35 (1) (1994 Jan) 104–107.
115. D. Vinereanu, B.P. Bajaj, J. Fenton-May, M.T. Rogers, C.F. Madler, A.G. Fraser, Subclinical cardiac involvement in myotonic dystrophy manifesting as decreased myocardial Doppler velocities, Neuromuscul. Disord. 14 (3) (2004 Mar) 188–194.
116. Freyermuth F, Rau F, Kokunai Y et al. Splicing misregulation of SCN5A contributes to cardiacconduction delay and heart arrhythmia in myotonic dystrophy. Nat Commun. 2016;7:11067.
117. Yadava RS, Frenzel-McCardell CD et al. RNA toxicity in myotonic muscular dystrophy induces NKX2-5 expression. Nat Genet. 2008;40:61–68
118. Rakocevic-Stojanovic V, Milovanovic B, Ivic N, Ille T, Marjanovic I, Stevic Z, Pavlovic S, Lavrnic D. Cardiac autonomic nervous system in patients with myotonic dystrophy type 1. Acta Myol. 2007;26:112–114
119. R.D. Leo, C. Rodolico, C.D. Gregorio, A. Recupero, S. Coglitore, G. Annesi, et al., Cardiovascular autonomic control in myotonic dystrophy type 1: a correlative study with clinical and genetic data, Neuromuscul. Disord. 14 (2) (2004) 136–141.
120. Lazarus A, Varin J, Jauvert G, Alonso C, Duboc D. Relationship between cardiac arrhythmias and sleep apnoea in permanently paced patients with type I myotonic dystrophy. Neuromuscul

121. Mandrioli J, Ferri L, Fasano A et al (2018) Cardiovascular diseases may play a negative role in the prognosis of amyotrophic lateral sclerosis. Eur J Neurol 25:861–868
122. Mazzoli M, Ariatti A, Garuti G, Agnoletto V, Fantini R, Marchioni A, Galassi G. Predictors of respiratory decline in myotonic dystrophy type 1 (DM1): a longitudinal cohort study. Acta Neurol Belg. 2021 Feb;121(1):133-142
123. Hermans MC, Faber CG et al. mStructural and functional cardiac changes in myotonic dystrophy type 1: a cardiovascular magnetic resonance study. J Cardiovasc Magn Reson. 2012;14:48.
124. Petri H, Ahtarovski KA et al. Myocardial fibrosis in patients with myotonic dystrophy type 1: a cardiovascular magnetic resonance study. J Cardiovasc Magn Reson. 2014;16:59.
125. D. Verhaert, K. Richards, J.A. Rafael-Fortney, S.V. Raman, Cardiac involvement in patients with muscular dystrophies: magnetic resonance imaging phenotype and genotypic considerations, Circ. Cardiovasc. Imaging 4 (1) (2011 Jan) 67–76
126. L. De Ambroggi, A. Raisaro, S. Marchiano, S. Radice, G. Meola, Cardiac involvement in patients with myotonic dystrophy: characteristic features of magnetic resonance imaging, Eur. Heart J. 16 (1995)
127. Poussel M1, Thil C2, Kaminsky P3, Mercy M4, Gomez E4, Chaouat A5, Chabot F5, Chenuel B6. Lack of correlation between the ventilatory response to CO2 and lung function impairment in myotonic dystrophy patients: evidence for a dysregulation at central level. Neuromuscul Disord. 2015 May;25(5):403-8.
128. Phillips MF1, Steer HM, Soldan JR, Wiles CM, Harper PS. Daytime somnolence in myotonic dystrophy. J Neurol. 1999 Apr;246(4):275-82.
129. Sansone VA, Gagnon C. Participants of the 207th ENMC workshop. 207th ENMC workshop on chronic respiratory insufficiency in myotonic dystrophies: management and implications for research, 27-29 June 2014, Naarden, The Netherlands.Neuromuscul Disord. 2015;25:432–42
130. Bégin P, Mathieu J, Almirall J, Grassino A. Relationship between chronic hypercapnia and inspiratory-muscle weakness in myotonic dystrophy. Am J Respir Crit Care Med 1997;156(1):133–9
131. Leonardis A, Maestri M, Nicoletta C, Vitrani G, Caccamo M, Siciliano G, Bonanni E, Centonze D, Sanduzzi A. Sleep Complaints, Sleep and Breathing Disorders in Myotonic Dystrophy Type 2. Curr Neurol Neurosci Rep 2019 Feb 9;19(2):9
132. Ono S, Kanda F, Takahashi K, Fukuoka Y, Jinnai K, Kurisaki H, Mitake S, Inagaki T, Nagao K. Neuronal loss in the medullary reticular formation in myotonic dystrophy: a clinicopathological study. Neurology. 1996 Jan;46(1):228
133. Poussel M1, Kaminsky P2, Renaud P3, Laroppe J3, Pruna L2, Chenuel B3 Supine changes in lung function correlate with chronic respiratory failure in myotonic dystrophy patients. Respir Physiol Neurobiol. 2014 Mar 1;193:43-51
134. Boentert M, Wenninger S, Sansone VA. Respiratory involvement in neuromuscular disorders. Curr Opin Neurol. 2017;30:529–37
135. Kierkegaard M, Harms-Ringdahl K, Holmqvist LW, Tollback A. Functioning and disability in adults with myotonic dystrophy type 1. Disabil Rehabil 2011;33(19-20):1826–36.
136. Kaminsky P, Poussel M, Pruna L, Deibener J, Chenuel B, Brembilla-Perrot B. Organ dysfunction and muscular disability in myotonic dystrophy type 1. Med (Baltim) 2011;90(4):262–8.
137. Arsenault ME, Prévost C, Lescault A, Laberge C, Puymirat J, Mathieu J. Clinical characteristics of myotonic dystrophy type 1 patients with small CTG expansions. Neurology 2006;66(8):1248–50
138. Leigh Hartog, Jing Zhao, Jerry Reynolds, Gabrielle Brokam, Ferdinand Vilson, W. David Arnold and Samantha LoRusso, Factors Influencing the Severity and progression of Respiratory Muscle Dysfunction in Myotonic Dystrophy Type 1- Front Neurol. 2021 Apr 13;12:658532.
139. Salvatore Rossi, Giacomo Della Marca, Martina Ricci, Alessia Perna, Tommaso F Nicoletti, Valerio Brunetti, Emiliana Meleo, Mariarosaria Calvello, Antonio Petrucci, Giovanni Antonini, Elisabetta Bucci, Loretta Licchelli, Cristina Sancricca, Roberto Massa, Emanuele Rastelli, Annalisa Botta, Antonio Di Muzio, Sonia Romano, Matteo Garibaldi, Gabriella Silvestri. Prevalence and predictor factors of respiratory impairment in a large cohort of patients with Myotonic Dystrophy type 1 (DM1): A retrospective, cross sectional study. J Neurol Sci 2019 Apr 15;399:118-124.
140. Seijger CG, Drost G, Posma JM, van Engelen BG, Heijdra YF. Overweight is an independent risk factor for reduced lung volumes in myotonic dystrophy Type 1. PLoS One 2016;11(3):e0152344.
141. Marchini C, Lonigro R, Verriello L, Pellizzari L, Bergonzi P, Damante G. Correlations between individual clinical manifestations and CTG repeat amplification in myotonic dystrophy. Clin Genet 2000;57(1):74–82.
142. Pruna L, Machado F, Louis L, Vasse G, Kaminsky P. [Muscular disability and organ impairments in myotonic dystrophy type 1]. Rev Neurol (Paris) 2011;167(1):23–8.
143. Marchini C, Lonigro R, Verriello L, Pellizzari L, Bergonzi P, Damante G. Correlations between individual clinical manifestations and CTG repeat amplification in myotonic dystrophy. Clin Genet 2000;57(1):74–82.
144. Maria Laura Ester Bianchi, Anna Losurdo, Chiara Di Blasi, Massimo Santoro, Marcella Masciullo, Giulia Conte, Venanzio Valenza, Antonello Damiani, Giacomo Della Marca, Gabriella Silvestri. Prevalence and clinical correlates of sleep disordered breathing in myotonic dystrophy types 1 and 2. Sleep Breath 2014 Sep;18
145. Leonardis L, Blagus R, Dolenc GL. Sleep and breathing disorders in myotonic dystrophy type 2. Acta Neurol Scand. 2015;132:42-48.
146. Bégin P, Mathieu J, Almirall J, Grassino A. Relationship between chronic hypercapnia and inspiratory-muscle weakness in myotonic dystrophy. Am J Respir Crit Care Med. 1997;156:133–9.

147. Simoncini C, Spadoni G, Lai E, Santoni L, Angelini C, Ricci G, Siciliano G. Central Nervous System Involvement as Outcome Measure for Clinical Trials Efficacy in Myotonic Dystrophy Type 1. Front Neurol. 2020 Oct 7;11:624. doi: 10.3389/fneur.2020.00624. eCollection 2020.
148. Kiyan E, Okumus G, Cuhadaroglu C, Deymeer F. Sleep apnea in adult myotonic dystrophy patients who have no excessive daytime sleepiness. Sleep Breath. 2010;14:19–24.
149. Montagnese F, Mondello S, Wenninger S, Kress W, Schoser B. Assessing the influence of age and gender on the phenotype of myotonic dystrophy type 2. J Neurol. 2017;264:2472–80 A detailed clinical chacterization of a large cohort of DM2 patients investigating the role of age and gender influencing DM2 phenotype.
150. Thil C, Agrinier N, Chenuel B, Poussel M. Longitudinal course of lung function in myotonic dystrophy type 1. Muscle Nerve2017;56(4):816–18.
151. Gagnon C, Heatwole C, Hébert LJ, Hogrel J-Y, Laberge L, Leone M, et al. Report of the third outcome measures in myotonic dystrophy type 1 (OMMYD-3) international workshop Paris, France,June 8, 2015. J Neuromuscul Dis. 2018;5
152. Matthias Boentert, Michelle Cao, Daphne Mass, Elisa De Mattia, Elisa Falcier, Miguel Goncalves, Venessa Holland,, Sherri Lynne Katz, David Orlikowski, Giulia Sannicolò, Peter Wijkstra, Leah Hellerstein, Valeria A Sansone. Consensus-Based Care Recommendations for Pulmonologists Treating Adults with Myotonic Dystrophy Type 1. Respiration. 2020;99(4):360-368.
153. Angelini C, Di Leo R, Cudia P. Autonomic regulation in muscular dystrophy. Front Physiol. 2013 Sep 20;4:257.
154. Di Leo R, Rodolico C et al. Cardiovascular autonomic control in myotonic dystrophy type 1: a correlative study with clinical and genetic data. Neuromuscul Disord. 2004 Feb;14(2):136-41.
155. Bienias P, Łusakowska A, Ciurzyński M, Rymarczyk Z, Irzyk K, Konwerski M, Ciąpała K, Kowalski P, Kamińska A, Pruszczyk P. Cardiac autonomic function in type 1 and type 2 myotonic dystrophy. Clin Auton Res. 2017 Jun;27(3):193-202.
156. Oloffson BO, Niklasson U, Forsberg H, Bjerle P, Andersson S, Henriksson A. Assessment of autonomic nerve function in myotonic dystrophy. J Auton Nerv Syst 1990;29:187–92.
157. Den Heijer JC, Van Dijk JG, Bollen WL, et al. Assessment of autonomic function in myotonic dystrophy. J Neurol Neurosurg Psychiatry 1991;54:531-4
158. Pierangeli G, Lugaresi A, Contin M, et al. Autonomic nervous system function in myotonic dystrophy. Ital J Neurol Sci 1992;13:589–92
159. Omboni S, Parati G, Di Rienzo M, Wieling W, Mancia G. Blood pressure and heart rate variability in autonomic disorders: a critical review. Clin Auton Res 1996;6:171–82.
160. Inoue K, Ogata H, Matsui M, et al. Assessment of autonomic function in myotonic dystrophy by spectral analysis of heart-rate variability. J Auton Nerv Syst 1995;55:131–4.
161. Hardin BA, Lowe MR, Bhakta D, Groh WJ. Heart rate variability declines with increasing age and CTG repeat length in patients with myotonic dystrophy type 1. AN E 2003;8:227–32
162. Rakocević-Stojanović V, Milovanović B et al. Cardiac autonomic nervous system in patients with myotonic dystrophy type 1. Acta Myol 2007 Oct;26(2):112-4
163. Flachenecker P, Schneider C, Cursiefen S, Ricker K, Toyka KV, Reiners K. Assessment of cardiovascular autonomic function in myotonic dystrophy type 2 (DM2/PROMM). Neuromuscul Disord 2003;13:289–93.
164. Magrì D, Piccirillo G. Increased temporal dispersion of myocardial repolarization in myotonic dystrophy type 1: beyond the cardiac conduction system. Int J Cardiol. 2012 May 3;156(3):259-64.
165. Salvatore Rossi, Angela Romano, Anna Modoni, Francesco Perna, Valentina Rizzo, Massimo Santoro, Mauro Monforte, Maurizio Pieroni, Marco Luigetti, Maria Grazia Pomponi, Gabriella Silvestri. Dysautonomia as Onset Symptom of Myotonic Dystrophy Type 2. Eur Neurol. 2018;79(3-4):166-170.
166. Tobaldini E, Colombo G et al. Cardiac autonomic control during sleep in patients with myotonic dystrophy type 1: the effects of comorbid obstructive sleep apnea Sleep. Medicine 39 (2017) 32e37
167. Andrea M Glaser, Jennifer H Johnston, Wallace A Gleason, J Marc Rhoads. Myotonic dystrophy as a cause of colonic pseudoobstruction: not just another constipated child. Clin Case Rep. 2015 Jun;3(6):424-6.
168. Bellini, M., S. Biagi, C. Stasi, F. Costa, M. G. Mumolo, A. Ricchiuti, et al. 2006. Gastrointestinal manifestations in myotonic muscular dystrophy. World J. Gastroenterol. 12:1821–1828.
169. Thompson H, Van Allen M, Von Noorden G. The pupil in myotonic dystrophy. Invest Ophtha/mo/1964; 3: 325-338.
170. Spaide R. Decreased sympathetic stimulation to the pupils in two patients with myotonic dystrophy. Ann Ophthalmol l.1984;3:685-8.
171. Alon M, Korczyn AD. Pupillary responses and blink reflex in myotonic dystrophy Clin Auton Res 199;2(1):17-9.
172. Peric M, Peric S, Rapajic N, Dobricic V, Savic-Pavicevic D, Nesic I, et al. Multidimensional aspects of pain in myotonic dystrophies. Acta Myol. 2015;34:126–32.
173. Hilbert JE, Ashizawa T, Day JW, et al. Diagnostic odyssey of patients with myotonic dystrophy. J Neurol 2013;260:2497-504.
174. George A, Schneider-Gold C, Zier S, Reiners K, Sommer C. Musculoskeletal pain in patients with myotonic dystrophy type 2. Arch Neurol. 2004;61:1938–42.
175. van Vliet J, Tieleman AA, Verrips A, Timmerman H, van Dongen RTM, van Engelen BGM, et al. Qualitative and quantitative aspects of pain in patients with myotonic dystrophy type 2. J Pain. 2018;19: 920–30 P

176. Rakocevic Stojanovic V, Peric S, Paunic T, Pesovic J, Vujnic M, Peric M, et al. Quality of life in patients with myotonic dystrophy type 2. J Neurol Sci. 2016;365:158–61 E
177. Tieleman AA, Jenks KM, Kalkman JS, Borm G, van Engelen BGM. High disease impact of myotonic dystrophy type 2 on physical and mental functioning. J Neurol. 2011;258:1820–6.
178. Moshourab R, Palada V, Grunwald S, Grieben U, Lewin GR, Spuler S. A molecular signature of myalgia in myotonic dystrophy2. EBioMedicine. 2016;7:205–11.
179. Boland-Freitas R, Ng K. Assessment of small sensory fiber function in myotonic dystrophy type 1. Muscle Nerve. 2019 Nov;60(5):575-579.
180. Hermans MC, Faber CG, Vanhoutte EK, Bakkers M, De Baets MH, de Die-Smulders CE, Merkies IS: Periphera l neuropathy in myotonic dystrophy type 1. J Peripher Nerv Syst 2011;16:24–29.
181. Peric S, Stojanovic VR, Nikolic A, Kacar A, Basta I, Pavlovic S, Lavrnic D: Peripheral neuropathy in patients with myotonic dystrophy type 1. Neurol Res 2013;35:331–335.
182. Bae JS, Kim OK, Kim SJ, Kim BJ. Abnormalities of nerve conduction studies in myotonic dystrophy type 1: primary involvement of nerves or incidental coexistence? J Clin Neurosci (2008) 15(10):1120–4.
183. Nojszewska M, Łusakowska A, SzmidtSalkowska E, Gaweł M, Lipowska M, Sułek A, Krysa W, Rajkiewicz M, Seroka A, Kaczmarek K, Kamińska AM: Peripheral nerve involvement in myotonic dystrophy type 2 – similar or different than in myotonic dystrophy type 1? Neurol Neurochir Pol 2015;49:164–170.
184. Leonardis L: Peripheral neuropathy in patients with myotonic dystrophy type 2. Acta Neurol Scand 2017;135:568–575.
185. Pagliarani E, Donadio V, Incensi S, De Pasqua S, Avoni P, Liguori R. Laser evoked potentials and skin biopsy to evaluate small nerve fiber dysfunction in myotonic dystrophy type 1 (DM1): a preliminary study. Clin Neurophysiol. 2017;128:e431.
186. Kuru S, Sakai M, Yoshida M, Mimuro M, Hashizume Y. Neurofibrillary tangles in the peripheral sympathetic ganglia of patients with myotonic dystrophy. Neuropathol Appl Neurobiol. 2013 Aug;39(5):585-8.
187. Ciafaloni E, Mignot E, Sansone V, Hilbert JE, Lin L, Lin X, et al. The hypocretin neurotransmission system in myotonic dystrophy type 1. Neurology. 2008;70:226–30.
188. Yu H, Laberge L, Jaussent I, Bayard S, Scholtz S, Raoul M, Pages M, Dauvilliers Y. Daytime sleepiness and REM sleep characteristics in myotonic dystrophy: a case-control study. Sleep. 2011 Feb 1;34(2):165-70.
189. Laberge L, Bégin P, Dauvilliers Y, Beaudry M, Laforte M, Jean S, Mathieu J A polysomnographic study of daytime sleepiness in myotonic dystrophy type 1. .J Neurol Neurosurg Psychiatry. 2009 Jun;80(6):642-6.
190. Romigi A, Izzi F, Pisani V, Placidi F, Pisani LR, Marciani MG, et al. Sleep disorders in adult-onset myotonic dystrophy type 1: a controlled polysomnographic study. Eur J Neurol. 2011;18:1139–45.
191. Romigi A, Albanese M, Liguori C, Placidi F, Marciani MG, Massa R. Sleep-wake cycle and daytime sleepiness in the myotonic dystrophies. J Neurodegener Dis 2013;2013:692026.
192. Giubilei F, Antonini G, Bastianello S, Morino S, Paolillo A, Fiorelli M, et al. Excessive daytime sleepiness in myotonic dystrophy. J Neurol Sci. 1999;164:60–3.
193. Martinez-Rodriguez JE, Lin L, Iranzo A, Genis D, Marti MJ, Santamaria J, et al. Decreased hypocretin-1 (orexin-A) levels in the cerebrospinal fluid of patients with myotonic dystrophy and excessive daytime sleepiness. Sleep. 2003;26:287–90.
194. Sub H Subramony , James P Wymer, Belinda S Pinto, Eric T Wang. Sleep disorders in myotonic dystrophies. Muscle Nerve. 2020 Sep;62(3):309-320.
195. van der Meché FG, Bogaard JM, van der Sluys JC, Schimsheimer RJ, Ververs CC, Busch HF. Daytime sleep in myotonic dystrophy is not caused by sleep apnoea. J Neurol Neurosurg Psychiatry. 1994;57:626–8.
196. Laberge L, Gagnon C, Dauvilliers Y.Daytime sleepiness and myotonic dystrophy. Curr Neurol Neurosci Rep. 2013 Apr;13(4):340. doi: 10.1007/s11910-013-0340-9.
197. Romigi A, Franco V, Placidi F, Liguori C, Rastelli E, Vitrani G, et al. Comparative sleep disturbances in myotonic dystrophy types 1 and 2. Curr Neurol Neurosci Rep. 2018;18:102
198. Tieleman AA, Knoop H, van de Logt A-E, Bleijenberg G, van Engelen BGM, Overeem S. Poor sleep quality and fatigue but no excessive daytime sleepiness in myotonic dystrophy type 2. J Neurol Neurosurg Psychiatry. 2010;81:963–7
199. Heatwole C, Johnson N, Bode R, Dekdebrun J, Dilek N, Hilbert JE, et al. Patient-reported impact of symptoms in myotonic dystrophy type 2 (PRISM-2). Neurology. 2015;85:2136–46
200. Rakocevic Stojanovic V, Peric S, Paunic T, Pesovic J, Vujnic M, Peric M, et al. Quality of life in patients with myotonic dystrophy type 2. J Neurol Sci. 2016;365:158–61
201. Enrica Bonanni, Luca Carnicelli, et al. Disruption of sleep-wake continuum in myotonic dystrophy type 1: Beyond conventional sleep stagingNeuromuscul Disord 2018 May;28(5):414-421.
202. Pincherle A, Patruno V, Raimondi P, Moretti S, Dominese A, MartinelliBoneschi F, et al. Sleep breathing disorders in Italian patients with Myotonic dystrophy type 1. Neuromuscul Disord. 2012;22:219–24.
203. Dauvilliers YA, Laberge L. Myotonic dystrophy type 1, daytime sleepiness and REM sleep dysregulation. Sleep Med Rev. 2012 Dec;16(6):539-45.
204. Y Dauvilliers, C. Baumann, B Carlander, M Bischof, T Blatter, M Lecendreux, F Maly, A Besset, J Touchon, M Billiard, M Tafti, C Bassetti. CSF hypocretin-1 levels in narcolepsy, Kleine-Levin syndrome, and other hypersomnias and neurological conditions J Neurol Neurosurg Psychiatry. 2003 Dec; 74(12): 1667–1673.

205. Shepard P, Lam EM, St Louis EK, Dominik J. Sleep disturbances in myotonic dystrophy type 2. Eur Neurol. 2012;68:377-380.
206. Lam EM, Shepard PW, St Louis EK, et al. Restless legs syndrome and daytime sleepiness are prominent in myotonic dystrophy type 2. Neurology. 2013;81:157-164.
207. Romigi A, Albanese M, Placidi F, et al. Sleep disorders in myotonic dystrophy type 2: a controlled polysomnographic study and selfreported questionnaires. Eur J Neurol. 2014;21:929-934
208. S1, Takahashi K, Jinnai K, Kanda F, Fukuoka Y, Kurisaki H, Mitake S, Inagaki T, Yamano T, Shimizu N, Nagao K. Loss of catecholaminergic neurons in the medullary reticular formation in myotonic dystrophy. Neurology. 1998 Oct;51(4):1121-4.
209. Timchenko LT. Myotonic dystrophy: the role of RNA CUG triplet repeats. Am J Hum Genet. 1999;64:360–4.
210. Schneider-Gold C, Bellenberg B, Prehn C, Krogias C, Schneider R, Klein J, 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:e0130352.
211. Wozniak JR, Mueller BA, Lim KO, Hemmy LS, Day JW. Tractography reveals diffuse white matter abnormalities in Myotonic Dystrophy Type 1. J Neurol Sci. (2014) 341:73–8.
212. Cabada T, Iridoy M, Jerico I, Lecumberri P, Seijas R, Gargallo A, et al. Brain involvement in myotonic dystrophy type 1: a morphometric and diffusion tensor imaging study with neuropsychological correlation. Arch Clin Neuropsychol. (2017) 32:401–12.
213. Coccagna G, Martinelli P, Lugaresi E. Sleep and alveolar hypoventilation in myotonic dystrophy. Acta Neurol Belg. 1982;82:185–94.
214. Finnimore AJ, Jackson RV, Morton A, Lynch E. Sleep hypoxia in myotonic dystrophy and its correlation with awake respiratory function. Thorax. 1994;49:66–70.
215. Bhat S, Sander HW, Grewal RP, Chokroverty S. Sleep disordered breathing and other sleep dysfunction in myotonic dystrophy type 2.Sleep Med. 2012;13:1207-1208.
216. Shepard P, Lam EM, St Louis EK, Dominik J. Sleep disturbances in myotonic dystrophy type 2. Eur Neurol. 2012;68:377-380.
217. Banach M, Antczak J, Rola R. Association of peripheral neuropathy with sleep-related breathing disorders in myotonic dystrophies. Neuropsychiatry Dis Treat. 2017;13:133-140.
218. Bosco G, Diamanti S, Meola G, Angeard N, Bassez G, Ekström AB, et al. Workshop report: consensus on biomarkers of cerebral involvement in yotonic dystrophy, 2-3 December 2014, Milan, Italy. Neuromusc Disord. (2015) 25:813–23.
219. Bugiardini E, Meola G, Alvarez C, Angeard N, Bassez G, Bugiardini E, et al. Consensus on cerebral involvement in myotonic dystrophy. Workshop report: May 24-27. 2013, Ferrere (AT), Italy. Neuromusc Disord. (2014) 24:445–52.
220. 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) 147:918–26.
221. Ekström AB, Hakenäs-Plate L, Tulinius M, et al. Cognition and adaptive skills in myotonic dystrophy type 1: a study of 55 individuals with congenital and childhood forms. Dev Med Child Neurol 2009;51: 982–90.
222. Modoni A, Silvestri G et al Characterization of the pattern of cognitive impairment in myotonic dystrophy type 1Arch Neurol. 2004 Dec;61(12):1943-7.
223. Angeard N, Jacquette A, Gargiulo M, Radvanyi H, Moutier S, Eymard B, et al. A new window on neurocognitive dysfunction in the childhood form of myotonic dystrophy type 1 (DM1). Neuromusc Disord. (2011) 21:468– 76.
224. Douniol M, Jacquette A, Cohen D, Bodeau N, Rachidi L, Angeard N, et al. Psychiatric and cognitive phe- notype of childhood myotonic dystrophy type 1. Dev Med Child Neurol. (2012) 54:905–11.
225. Lindeblad G, Kroksmark AK, Ekström AB. Cognitive and adaptive functioning in congenital and childhood forms of myotonic dystrophy type 1: a longitudinal study. Dev Med Child Neurol. (2019) 61:1214– 20.
226. Douniol M, Jacquette A et al. Psychiatric and cognitive phenotype in children and adolescents with myotonic dystrophy Eur Child Adolesc Psychiatry. 2009 Dec;18(12):705-15
227. Steyaert J, Umans S, Willekens D, Legius E, Pijkis E, De Die-Smulders C et al. (1997) A study of the cognitive and psychological profile in 16 children with congenital or juvenile myotonic dystrophy. Clin Genet 52(3):135–141.
228. Goossens E, Steyaert J, De Die-Smulders C, Willekens D, Fryns JP (2000) Emotional and behavioural profile and child psychiatric diagnosis in the childhood type of myotonic dystrophy. Genet Couns 11:317–327
229. Echenne B, Rideau A, Roubertie A, Se´bire G, Rivier F, Lemieux B (2008) Myotonic dystrophy type I in childhood: long-term evolution in patients surviving the neonatal period. Eur J Paediatr Neurol 12(3):210–223
230. Cohen D, Plaza M, Angeard N, Lanthier-Gazzano O, Baraud P, Rivie`re JP et al (2006) Reading and spelling impairments in children and adolescents with infantile myotonic dystrophy. J Neurolinguistic 19(6):455–465
231. Woo J, Lee HW, Park JS. Differences in the pattern of cognitive impairments between juvenile and adult onset myotonic dystrophy type 1. J Clin Neurosci. (2019) 68:92–6.
232. Jean S, Richer L, Laberge L, Mathieu J. Comparisons of intellectual capacities between mild and classic adult-onset phenotypes of myotonic dystrophy type 1 (DM1). Orphanet J Rare Dis. (2014) 9:186.

233. Modoni A, Silvestri G, Pomponi MG, Mangiola F, Tonali PA, Marra C. Characterization of the pattern of cognitive impairment in myotonic dystrophy type 1. Arch Neurol (2004) 61(12):1943–7.
234. Winblad S, Lindberg C, Hansen S. Cognitive deficits and CTG repeat expansion size in classical myotonic dystrophy type 1 (DM1). Behav Brain Funct (2006) 2:16.
235. Sugiyama A, Sone D, Sato N, Kimura Y, Ota M, Maikusa N, et al. Brain gray matter structural network in myotonic dystrophy type 1. PLoS One (2017) 12(11):e0187343.
236. Minnerop M, Weber B, Schoene-Bake JC, Roeske S, Mirbach S, Anspach C, et al. The brain in myotonic dystrophy 1 and 2: evidence for a predominant white matter disease. Brain (2011) 134(Pt 12):3530–46.
237. Caso F, Agosta F, Peric S, Rakocevic-Stojanovic V, Copetti M, Kostic VS, et al. Cognitive impairment in myotonic dystrophy type 1 is associated with white matter damage. PLoS ONE (2014) 9:e104697.
238. Gallais B, Gagnon C, Mathieu J, Richer L. Cognitive decline over time in adults with myotonic dystrophy type 1: a 9-year longitudinal study. Neuromusc Disord. (2017) 27:61–72.
239. Callus E, Bertoldo EG, Beretta M, Boveri S, Cardani R, Fossati B, et al. Neuropsychological and psychological functioning aspects in Myotonic Dystrophy Type 1 patients in Italy. Front Neurol. (2018) 9:751.
240. Meola G, Sansone V. Cerebral involvement in myotonic dystrophies. Muscle Nerve (2007) 36:294–306.
241. Baldanzi S, Cecchi P, Fabbri S, Pesaresi I, Simoncini C, Angelini C, et al. Relationship between neuropsychological impairment and grey and white matter changes in adult-onset myotonic dystrophy type 1. Neuroimage Clin.
242. Baldanzi S, Bevilacqua F, Lorio R, Volpi L, Simoncini C, Petrucci A, et al. Disease awareness in myotonic dystrophy type 1: an observational cross-sectional study. Orphanet J Rare Dis. (2016) 11:34.
243. Schneider-Gold C, Bellenberg B, Prehn C, Krogias C, Schneider R, Klein J, 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:e0130352.
244. Winblad S, Samuelsson L, Lindberg C, Meola G. Cognition in myotonic dystrophy type 1: a 5-year follow-up study. Eur J Neurol. (2016) 23:1471–
245. Labayru G, Aliri J, Zulaica M, López de Munain A, Sistiaga A (2020) Age-related cognitive decline in myotonic dystrophy type 1: an 11-year longitudinal follow-up study. J Neuropsychol 14: 121–134
246. 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
247. Modoni A, Silvestri G, Gabriella Vita M, Quaranta D, Tonali PA, Marra C (2008) Cognitive impairment in myotonic dystrophy type 1 (DM1). A longitudinal follow-up study. J Neurol 255:1737–1742
248. Jean S, Richer L, Laberge L, Mathieu J. Comparisons of intellectual capacities between mild and classic adult-onset phenotypes of myotonic dystrophy type 1 (DM1). Orphanet J Rare Dis. (2014) 9:186.
249. J C den Heijer 1, J G van Dijk, W L Bollen, J E Bos, A R Wintzen. J Neurol Neurosurg Psychiatry 1991 Jun;54(6):531-4. doi: 10.1136/jnnp.54.6.531. Assessment of autonomic function in myotonic dystrophy
250. Bertrand JA, Jean S, Laberge L, Gagnon C, Mathieu J, Gagnon JF, et al. Psychological characteristics of patients with myotonic dystrophy type 1. Acta Neurol Scand. (2015) 132:49–58.
251. 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:e0156901.
252. Serra1 L, Bianchi G et al Abnormal Cortical Thickness Is Associated With Deficits in Social Cognition in Patients With Myotonic Dystrophy Type 1. Front. Neurol., 28 February 2020
253. Wozniak JR, Mueller BA, Bell CJ, Muetzel RL, Lim KO, Day JW. Diffusion tensor imaging reveals widespread white matter abnormalities in children and adolescents with myotonic dystrophy type 1. J Neurol. (2013) 260:1122–31.
254. Wozniak JR, Mueller BA, Lim KO, Hemmy LS, Day JW. Tractography reveals diffuse white matter abnormalities in Myotonic Dystrophy Type 1. J Neurol Sci. (2014) 341:73–8.
255. Itoh K, Mitani M et al. Neuropathology does not Correlate with Regional Differences in the Extent of Expansion of CTG Repeats in the Brain with Myotonic Dystrophy Type 1 Histochem Cytochem . 2010 Dec 29;43(6):149-56.
256. Caillet-Boudin ML, Fernandez-Gomez FJ, Tran H, et al. Brain pathology in myotonic dystrophy: when Tauopathy meets spliceopathy and RNAopathy. Front Mol Neurosci 2014;6(57):1–20.
257. Goodwin M, Mohan A, Batra R, Lee KY, Charizanis K, Fernandez Gomez FJ, et al. MBNL sequestration by toxic RNAs and RNA misprocessing in the myotonic dystrophy brain. Cell Rep (2015) 12(7):1159–68.
258. Winblad S, Ma˚nsson JE, Blennow K, Jensen C, Samuelsson L, Lindberg C. Cerebrospinal fluid tau and amyloid beta42 protein in patients with myotonic dystrophy type 1. Eur J Neurol 2008;15(9):947–52.
259. Peric S, Mandic-Stojmenovic G, Markovic I. Cerebrospinal fluid biomarkers of neurodegeneration in patients with juvenile and classic myotonic dystrophy type 1. Eur J Neurol 2014;21:231–7.
260. Wansink DG, Gourdon G, van Engelen BG, Schoser B. 248th ENMC international workshop: Myotonic dystrophies: molecular approaches for clinical purposes, framing a European molecular research network Hoofddorp, The Netherlands, 11–13 October 2019. Neuromusc Disord.(2020) 30:521–31.
261. Okkersen K, Monckton DG, Le N, Tuladhar AM, Raaphorst J, van Engelen BGM. Brain imaging in myotonic dystrophy type 1: a systematic review. Neurology (2017) 89:960–9.

262. Ekstrom AB, Tulinius M, Sjostrom A, Aring E. Visual function in congenital and childhood myotonic dystrophy type 1. Ophthalmology (2010) 117(5):976–82.
263. Abed E, D'Amico G, Rossi S, Perna A, Bianchi MLE, Silvestri G. Spectral domain optical coherence tomography findings in myotonic dystrophy. Neuromuscul Disord. 2020 Feb;30(2):144-150.
264. Passeri E, Bugiardini E, Sansone VA, Valaperta R, Costa E, Ambrosi B, et al. Vitamin D, parathyroid hormone and muscle impairment in myotonic dystrophies. J Neurol Sci (2013) 331(1–2):132–5.
265. Terracciano C, Rastelli E, Morello M, Celi M, Bucci E, Antonini G, et al. Vitamin D deficiency in myotonic dystrophy type 1. J Neurol (2013) 260(9):2330–4.
266. Dahlqvist JR, Orngreen MC, Witting N, Vissing J. Endocrine function over time in patients with myotonic dystrophy type 1. Eur J Neurol (2015) 22(1):116–22.
267. Ronnemaa T, Alaranta H, Viikari J, Tilvis R, Falck B. Increased activity of serum gamma-glutamyltransferase in myotonic dystrophy. Acta Med Scand (1987) 222(3):267–73.
268. Kalafateli M, Triantos C, Tsamandas A, Kounadis G, Labropoulou-Karatza C. Abnormal liver function tests in a patient with myotonic dystrophy type 1. Ann Hepatol (2012) 11(1):130–3.
269. Brunner HG, Hamel BC, Rieu P, Howeler CJ, Peters FT. Intestinal pseudoobstruction in myotonic dystrophy. J Med Genet (1992) 29(11):791–3.
270. Bellini M, Biagi S, Stasi C, Costa F, Mumolo MG, Ricchiuti A, et al. Gastrointestinal manifestations in myotonic muscular dystrophy. World J Gastroenterol (2006) 12(12):1821–8.
271. Goldberg HI, Sheft DJ. Esophageal and colon changes in myotonia dystrophica. Gastroenterology (1972) 63(1):134–9.
272. Gadalla SM, Lund M, Pfeiffer RM, Gortz S, Mueller CM, Moxley RT III, et al. Cancer risk among patients with myotonic muscular dystrophy. JAMA(2011) 306(22):2480–6.
273. Win AK, Perattur PG, Pulido JS, Pulido CM, Lindor NM. Increased cancer risks in myotonic dystrophy. Mayo Clin Proc (2012) 87(2):130–5.
274. Alsaggaf R, Wang Y, Marini-Bettolo C, Wood L, Nikolenko N, Lochmuller H, et al. Benign and malignant tumors in the UK myotonic dystrophy patient registry. Muscle Nerve (2018) 57(2):316–20.
275. Franc DT, Muetzel RL, Robinson PR, Rodriguez CP, Dalton JC, Naughton CE, et al. Cerebral and muscle MRI abnormalities in myotonic dystrophy. Neuromuscul Disord. (2012) 22:483–91.
276. Ates S, Deistung A, Schneider R, Prehn C, Lukas C, Reichenbach JR, et al. Characterization of iron accumulation in deep grey matter in Myotonic Dystrophy type 1 and 2 using quantitative susceptibility mapping and R2∗ relaxometry: a magnetic resonance imaging study at 3 Tesla. Front Neurol. (2019) 10:1320.
277. Antonini G, Mainero C, Romano A, Giubilei F, Ceschin V, Gragnani F, et al. Cerebral atrophy in myotonic dystrophy: a voxel based morphometric study. J Neurol Neurosurg Psychiatry. (2004) 75:1611– 3.
278. Gliem C, Minnerop M, Roeske S, Gärtner H, Schoene-Bake JC, Adler S, Witt JA, Hoffstaedter F, Schneider-Gold C, Betz RC, 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: e0213381
279. Zanigni S, Evangelisti S, Giannoccaro MP, Oppi F, Poda R, Giorgio A, et al. Relationship of white and gray matter abnormalities to clinical and genetic features in myotonic dystrophy type 1. Neuroimage Clin. (2016) 11:678–85.
280. brain/awr299
281. Weber YG, Roebling R, Kassubek J, Hoffmann S, Rosenbohm A, Wolf M, et al. Comparative analysis of brain structure, metabolism, and cognition in myotonic dystrophy 1 and 2. Neurology (2010) 74:1108–17.
282. Serra L, Petrucci A, Spano B, Torso M, Olivito G, Lispi L, et al. How genetics affects the brain to produce higher-level dysfunctions in myotonic dystrophy type 1. Funct Neurol. (2015) 30:21–31.
283. Cabada T, Iridoy M, Jerico I, Lecumberri P, Seijas R, Gargallo A, et al. Brain involvement in myotonic dystrophy type 1: a morphometric and diffusion tensor imaging study with neuropsychological correlation. Arch Clin Neuropsychol. (2017) 32:401–12.
284. Romeo V, Pegoraro E, Ferrati C, Squarzanti F, Soraru G, Palmieri A, et al. Brain involvement in myotonic dystrophies: neuroimaging and neuropsychological comparative study in DM1 and DM2. J Neurol. (2010) 257:1246–55. doi: 10.1007/s00415-010-5498-3
285. van der Plas E, Hamilton MJ, Miller JN, Koscik TR, Long JD, Cumming S, et al. Brain structural features of myotonic dystrophy type 1 and their relationship with CTG repeats. J Neuromusc Dis. 6:321–32.
286. Minnerop M, Gliem C, Kornblum C. Current Progress in CNS Imaging of Myotonic Dystrophy. Front Neurol 2018 Aug 21;9:646
287. Ota M, Sato N, Ohya Y, Aoki Y, Mizukami K, Mori T, et al. Relationship between diffusion tensor imaging and brain morphology in patients with myotonic dystrophy. Neurosci Lett. (2006) 407:234–9.
288. Fukuda H, Horiguchi J, Ono C, Ohshita T, Takaba J, Ito K. Diffusion tensor imaging of cerebral white matter in patients with myotonic dystrophy. Acta Radiologica (2016) 46:104–9.
289. Naka H, Imon Y, Ohshita T, Honjo K, Kitamura T, Mimori Y, et al. Magnetization transfer measurements of cerebral white matter in patients with myotonic dystrophy. J Neurol Sci. (2002) 193:111–6.
290. Di Costanzo A, Di Salle F, Santoro L, Bonavita V, Tedeschi G. T2 relaxometry of brain in myotonic dystrophy. Neuroradiology (2001) 43:198–204.

291. Wozniak JR, Mueller BA, Ward EE, Lim KO, Day JW. White matter abnormalities and neurocognitive correlates in children and adolescents with myotonic dystrophy type 1: a diffusion tensor imaging study. Neuromuscul Disord. (2011) 21:89–96.
292. Park JS, Song H, Jang KE, Cha H, Lee SH, Hwang SK, et al. Diffusion tensor imaging and voxel-based morphometry reveal corticospinal tract involvement in the motor dysfunction of adult-onset myotonic dystrophy type 1. Sci Rep. (2018) 8:1–11.
293. Cabada T, Díaz J et al. Longitudinal study in patients with myotonic dystrophy type 1: correlation of brain MRI abnormalities with cognitive performances Neuroradiology. 2021 Jul;63(7):1019-1029
294. 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. Neuroradiol J 29:36–45
295. Labayru G, Diez I et al. Regional brain atrophy in gray and white matter is associated with cognitive impairment in Myotonic Dystrophy type 1. Neuroimage Clin. 2019;24:102078. doi: 10.1016/j.nicl.2019.102078. Epub 2019 Nov 6.
296. Serra L, Petrucci A, Spano B, Torso M, Olivito G, Lispi L, et al. How genetics affects the brain to produce higher-level dysfunctions in myotonic dystrophy type 1. Funct Neurol. (2015) 30:21–31.
297. Toth A, Lovadi E, Komoly S, Schwarcz A, Orsi G, Perlaki G, et al. Cortical involvement during myotonia in myotonic dystrophy: an fMRI study. Acta Neurol Scand. (2015) 132:65-72.
298. Contardi, S., Pizza, F., Falzone, F., et al., 2012. Development of a disability scale for myotonic dystrophy type 1. Acta Neurol. Scand. 125 (6), 431–438.
299. Weber YG, Roebling R, Kassubek J, Hoffmann S, Rosenbohm A, Wolf M, et al. Comparative analysis of brain structure, metabolism, and cognition in myotonic dystrophy 1 and 2. Neurology (2010) 74:1108–17. Doi 10.1212/WNL.0b013e3181d8c35f
300. van der Velden BG, Okkersen K, Kessels RP, Groenewoud J, van Engelen B, Knoop H, Raaphorst J. Affective symptoms and apathy in myotonic dystrophy type 1 a systematic review and meta-analysis. J Affect Disord. 2019;250:260–9. [PubMed: 30870776]
301. Peric S, Brajkovic L, Belanovic B, Ilic V, Salak-Djokic B, Basta I, et al. Brain positron emission tomography in patients with myotonic dystrophy type 1 and type 2. J Neurol Sci. (2017) 378:187–92. doi: 10.1016/j.jns.2017.05.013
302. Weber YG, Roebling R, Kassubek J, Hoffmann S, Rosenbohm A, Wolf M, et al. Comparative analysis of brain structure, metabolism, and cognition in myotonic dystrophy 1 and 2. Neurology (2010) 74:1108–17.
303. Romeo V, Pegoraro E, Squarzanti F, Soraru G, Ferrati C, Ermani M, et al. Retrospective study on PET-SPECT imaging in a large cohort of myotonic dystrophy type 1 patients. Neurol Sci. (2010) 31:757–63.
304. Chang L, Ernst T, Osborn D, Seltzer W, Leonido-Yee M, Poland RE. Proton spectroscopy in myotonic dystrophy: correlations with CTG repeats. Arch Neurol. (1998)
305. Akiguchi I, Nakano S, Shiino A, Kimura R, Inubushi T, Handa J, et al. Brain proton magnetic resonance spectroscopy and brain atrophy in myotonic dystrophy. Arch Neurol. (1999) 56:325–30.
306. Vielhaber S, Jakubiczka S, Gaul C, Schoenfeld MA, Debska-Vielhaber G, Zierz S, et al. Brain 1H magnetic resonance spectroscopic differences in myotonic dystrophy type 2 and type 1. Muscle Nerve (2006) 34:145–52.
307. Takado Y, Terajima K, Ohkubo M, Okamoto K, Shimohata T, Nishizawa M, et al. Diffuse brain abnormalities in myotonic dystrophy type 1 detected by 3.0 T proton magnetic resonance spectroscopy. Eur Neurol. (2015) 73:247–56.
308. Caramia F, Mainero C, Gragnani F, Tinelli E, Fiorelli M, Ceschin V, et al. Functional MRI changes in the central motor system in myotonic dystrophy type 1. Magn Reson Imaging (2010) 28:226–34.
309. Serra L, Silvestri G, Petrucci A, Basile B, Masciullo M, Makovac E, et al. Abnormal functional brain connectivity and personality traits in myotonic dystrophy type 1. JAMA Neurol. (2014) 71:603–11.
310. Bullmore E, Sporns O. Complex brain networks: graph theoretical analysis of structural and functional systems. Nat Rev Neurosci. (2009) 10:186–98.
311. Serra L, Mancini M, Silvestri G, Petrucci A, Masciullo M, Spano B, et al. Brain connectomics' modification to clarify motor and nonmotor features of myotonic dystrophy type 1. Neural Plast. (2016) 2016:2696085.
312. Park JS, Seo J, Cha H, Song HJ, Lee SH, Jang KE, et al. Altered power spectral density in the resting-state sensorimotor network in patients with myotonic dystrophy type 1. Sci Rep. (2018) 8:987.
313. Liu C, Li W, Johnson GA, Wu B. High-field (9.4 T) MRI of brain dysmyelination by quantitative mapping of magnetic susceptibility. Neuroimage. (2011) 56:930–8.
314. Schweser F, Deistung A, Lehr BW, Reichenbach JR. Differentiation between diamagnetic and paramagnetic cerebral lesions based on magnetic susceptibility mapping. Med Phys. (2010) 37:5165–78.
315. Ates S, Deistung A, Schneider R, Prehn C, Lukas C, Reichenbach JR, et al. Characterization of iron accumulation in deep grey matter in Myotonic Dystrophy type 1 and 2 using quantitative susceptibility mapping and R2∗ relaxometry: a magnetic resonance imaging study at 3 Tesla. Front Neurol. (2019) 10:1320.
316. Vihola A, Sirito M, Bachinski LL, Raheem O, Screen M, Suominen T, et al. Altered expression and splicing of Ca(2+) metabolism genes in myotonic dystrophies DM1 and DM2. Neuropathol Appl Neurobiol. (2013) 39:390–405.

317. Hlaing PM, Scott IA, Jackson RV. Dysregulation of calcium metabolism in type 1 myotonic dystrophy. Intern Med J. (2019) 49:1412–7.
318. Lam EM, Shepard PW, St. Louis EK, Dueffert LG, Slocumb N, McCarter SJ, et al. Restless legs syndrome and daytime sleepiness are prominent in myotonic dystrophy type 2. Neurology. (2013) 81:157–64.
319. Goodwin M, Mohan A, Batra R, Lee K-Y, Charizanis K, Fernández. Gómez FJ, et al. MBNL sequestration by toxic RNAs and RNA misprocessing in the myotonic dystrophy brain. Cell Rep. (2015) 12:1159–68.
320. Maurage CA, Udd B, Ruchoux MM, Vermersch P, Kalimo H, Krahe R, et al. Similar brain tau pathology in DM2/PROMM and DM1/Steinert disease. Neurology. (2005) 65:1636–8.
321. Wi´sniewski HM, Berry K, Spiro AJ. Ultrastructure of thalamic neuronal inclusions in myotonic dystrophy. J Neurol Sci. (1975) 24:321–9.
322. Oyanagi K, Ogawa H, Nakajima T. Rod-like intracytoplasmic inclusions in large neurons of the caudate nucleus: frequent appearance in myotonic dystrophy. Clin Neuropathol. (1994) 13:134–8
323. Schmaal L, Veltman DJ, van Erp TGM, Sämann PG, Frodl T, Jahanshad N, et al. Subcortical brain alterations in major depressive disorder: findings from the ENIGMA Major Depressive Disorder working group. Mol Psychiatry. (2016) 21:806–12.
324. Haider L, Simeonidou C, Steinberger G, Hametner S, Grigoriadis N, Deretzi G, et al. Multiple sclerosis deep grey matter: the relation between demyelination, neurodegeneration, inflammation and iron. J Neurol Neurosurg Psychiatry. (2014) 85:1386–95.
325. Stocco A, Lebiere C, Anderson JR. Conditional routing of information to the cortex: a model of the Basal Ganglia's role in cognitive coordination. Psychol Rev. (2010) 117:541–74. doi: 10.1037/a0019077
326. Houk JC, Wise SP. Distributed modular architectures linking basal ganglia, cerebellum, and cerebral cortex: their role in planning and controlling action. Cereb Cortex. (1995) 5:95–110.
327. Stoffers D, Altena E, van der Werf YD, Sanz-Arigita EJ, Voorn TA, Astill RG, et al. The caudate: a key node in the neuronal network imbalance of insomnia? Brain. (2014) 137:610–20. ì
328. Plante DT, Birn RM, Walsh EC, Hoks RM, Cornejo MD, Abercrombie HC. Reduced resting-state thalamostriatal functinal connectivity is associated with excessive daytime sleepiness in persons with and without depressive disorders. J Affect Disord. (2018) 227:517–20. ì
329. Macey PM, Prasad JP, Ogren JA, Moiyadi AS, Aysola RS, Kumar R, Yan-Go FL,Woo MA, Albert Thomas M, Harper RM. Sex-specific hippocampus volume changes in obstructive sleep apnea. Neuroimage Clin. 2018;20:305–17. [PubMed: 30101062]
330. Nobis WP, Schuele S, Templer JW, Zhou G, Lane G, Rosenow JM, Zelano C. Amygdalastimulation-induced apnea is attention and nasal-breathing dependent. Ann Neurol. 2018;83:460– [PubMed: 29420859]
331. Ono S, Takahashi K, Jinnai K, et al. Loss of serotonin-containing neurons in the raphe of patients with myotonic dystrophy: aquantitative immunohistochemical study and relation to hypersomnia. Neurology 1998;50:535–8.
332. Ono S, Kurisaki H, Sakuma A, Nagao K. Myotonic dystrophy with alveolar hypoventilation and hypersomnia: a clinicopathological study. J Neurol Sci. 1995;128:225–31.
333. P. D. H. Frens, "Hypersomnia associated with alveolar hypoventilation in myotonic dystrophy," Neurology, vol. 31, no. 10, pp.1336–1337, 1981.
334. F. Cirignotta, S. Mondini, M. Zucconi et al., "Sleep-related breathing impairment in myotonic dystrophy," Journal of Neurology, vol. 235, no. 2, pp. 80–85, 1987.
335. L. Laberge and Y. Dauvilliers, "Myotonic dystrophy and sleepiness," in Sleepiness: Causes, Consequences, Disorders and Treatment, M. Thorpy and M. Billiard, Eds., pp. 316–328, Cambridg University Press, Cambridge, UK, 2011.
336. G. Meola, V. Sansone, D. Perani et al., "Executive dysfunction and avoidant personality trait in myotonic dystrophy type 1 (DM-1) and in proximal myotonic myopathy (PROMM/DM-" Neuromuscular Disorders, vol. 13, no. 10, pp. 813–821, 2003.
337. S. Winblad, C. Lindberg, and S. Hansen, "Temperament and character in patients with classical myotonic dystrophy type 1 (DM-1)," Neuromuscular Disorders, vol. 15, no. 4, pp. 287–292, 2005.
338. Laberge L, Dauvilliers Y. Myotonic dystrophy and sleepiness. In: Thorpy M, Billiard M, editors. Sleepiness: causes, consequences, disorders and treatment. Cambridge, UK: Cambridge University Press; 2011. p. 316e28.
339. M. H. Pennestri, J. Montplaisir, R. Colombo, G. Lavigne, and P. A. Lanfranchi, "Nocturnal blood pressure changes in patients with restless legs syndrome," Neurology, vol. 68, no. 15, pp. 1213–1218, 2007.
340. A. S. Walters and D. B. Rye, "Evidence continues to mount on the relationship of restless legs syndrome/periodic limb
movements in sleep to hypertension, cardiovascular disease, and stroke," Sleep, vol. 33, no. 3, p. 287, 2010.
341. Laberge L, Dauvilliers Y, Bégin P, Richer L, Jean S, Mathieu J. Fatigue and daytime sleepiness in patients with myotonic dystrophy type 1: to lump or split? Neuromuscul Disord 2009;19:397e402.
342. Gourdon G, Meola G. Myotonic Dystrophies: State of the Art of New Therapeutic Developments for the CNS. Front Cell Neurosci. 2017 Apr 20;11:101. doi: 10.3389/fncel.2017.00101. eCollection 2017.
343. Okkersen K, Buskes M et al. The cognitive profile of myotonic dystrophy type 1: A systematic review and meta-analysisC ortex 2017 Oct;95:143-155. doi: 10.1016/j.cortex.2017.08.008. Epub 2017 Aug 16.

344. Rakocevic-Stojanovic V, Peric S, Madzarevic R, Dobricic V, Ralic V, Ilic V, et al. Significant impact of behavioral and cognitive impairment on quality of life in patients with myotonic dystrophy type 1. Clin Neurol Neurosurg. (2014) 126:76–81.
345. Winblad S, Hellström P, Lindberg C, Hansen S. Facial emotion recognition in myotonic dystrophy type 1 correlates with CTG repeat expansion. J Neurol Neurosurg Psychiatry. (2006) 77:219–23.
346. Takeda A, Kobayakawa M, Suzuki A, Tsuruya N, Kawamura M. Lowered sensitivity to facial emotions in myotonic dystrophy type 1. J Neurol Sci. (2009) 280:35–9.
347. 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. (2010) 290:48–51.
348. Gallais B, Montreuil M, Gargiulo M, Eymard B, Gagnon C, Laberge L. Prevalence and correlates of apathy in myotonic dystrophy type 1. BMC Neurol. (2015) 15:148.
349. Labayru G, Arenzana I, Aliri J, Zulaica M, de Munain AL. Social cognition in myotonic dystrophy type 1: specific or secondary impairment? PLoS ONE.(2018) 13:e0204227.
350. Fleming SM, Dolan RJ. The neural basis of metacognitive ability. Philos Trans R Soc B Biol Sci. (2012) 367:1338–49.
351. Prigatano GP, (editor). The Study of Anosognosia. Oxford University Press (2010).
352. Schmahmann JD. The role of the cerebellum in cognition and emotion: personal reflections since 1982 on the dysmetria of thought hypothesis, and its historical evolution from theory to therapy. Neuropsychol Rev. (2010) 20:236–60.
353. Folstein MF, Folstein SE, Mchugh PR (1975) ‘‘Mini-Mental State’’. A practical method for grading the cognitive state of patients for the clinician. J Psychiatr Res 12:189–198
354. Raven JC. Matrix test. Mental Health 1940; 1: 27-34
355. Kaplan E, Goodglass H, Weintraub S. (1983). Boston Naming Test. Philadelphia, PA: Lea & Febiger; 1983.
356. Wendy J. Mack, David M. Freed, Betsy White Williams, Victor W. Henderson, Boston Naming Test: Shortened Versions for Use in Alzheimer's Disease, Journal of Gerontology, Volume 47, Issue 3, May 1992, Pages P154–P158
357. Carlesimo G. A., Caltagirone C., Gainotti G., Fadda L., Gallassi R., Lorusso S., et al. (1996). The mental deterioration battery: normative data, diagnostic reliability and qualitative analyses of cognitive impairment. Eur. Neurol. 36 378–384. 10.1159/000117297
358. Novelli G, Papagno C, Capitani E, Laiacona N, Vallar G, et al. (1986) Tre test clinici di ricerca e produzione lessicale. Taratura su soggetti normali. Archivio di Psicologia, Neurologia e Psichiatria 47:477–506
359. Milner B (1971) Interhemispheric differences in the localization of psychological processes in man. Br Med Bull 27:272–277
360. Spinnler H, Tognoni G (1987) Standardizzazione e taratura italiana di test neuropsicologici. Ital J Neurol Sci 8[Suppl]:1–120
361. Carlesimo, G. A., Buccione, I., Fadda, L., Graceffa, A., Mauri, M., Lorusso, S., Bevilacqua, G., & Caltagirone, C. (2002). Standardizzazione di due test di memoria per uso clinico: Breve Racconto e Figura di Rey. Nuova Rivista di Neurologia, 12(1), 1-13.
362. Caffarra, P., Vezzadini, G., Dieci, F., Zonato, F., Venneri, A., 2002. Rey-Osterrieth complex figure: normative values in an Italian population sample. Neurol. Sci. 22 (6), 443–447.
363. Dubois B, Slachevsky A, Litvan I, Pillon B (2000) The FAB: a frontal assessment battery at bedside. Neurology 55: 1621-1626
364. Appollonio I, Piamarta F, Isella V, Leone M, Consoli T, Nichelli P (2004) Strumenti di lavoro: la Frontal Assessment Battery (FAB). Demenze 7: 28–33
365. Orsini A, Laicardi C (1998) WAIS-R Contributo alla Taratura Italiana. Giunti Organizzazioni Speciali Editore
366. Reitan, R. M., & Wolfson, D. (1985). The Halstead–Reitan Neuropsycholgical Test Battery: Therapy and clinical interpretation. Tucson, AZ: Neuropsychological Press.
367. Beck AT, Steer RA, Brown GK. BDI-II: Beck Depression Inventory Manual. 2nd ed. San Antonio: Psychological Corporation; 1996.
368. Spielberger, C. D. (1983). Manual for the State-Trait Anxiety Inventory (Form Y). Palo Alto: Consulting Psychologist Press.
369. Nucci, M., Mapelli, D., & Mondini, S. (2012). The cognitive Reserve Questionnaire (CRIq): a new instrument for measuring the cognitive reserve. Aging clinical and experimental research, 24, 218-126.
370. Apolone G, Mosconi P. The Italian SF-36 Health Survey: translation, validation and norming. J Clin Epidemiol. 1998 Nov;51(11):1025-36. doi: 10.1016/s0895-4356(98)00094-8. PMID: 9817120.
371. Rey, A. (1964). L ‘examen clinique en psychologie [Clinical tests in psychology]. Paris: Presses Universitaires de France
372. A.L Benton, N.R Varney, K.S Hamsher (1978) Visuospatial judgment. Archives of Neurology, 35 , pp. 364-367
373. Nocentini U, Giordano A, Di Vincenzo S, Panella M, Pasqualetti P. The Symbol Digit Modalities Test - Oral version: Italian normative data. Funct Neurol. 2006 Apr-Jun;21(2):93-6. PMID: 16796824

374. Baron-Cohen S, Wheelwright S, Hill J, Raste Y, Plumb I. The "Reading the Mind in the Eyes" Test revised version: a study with normal adults, and adults with Asperger syndrome or high-functioning autism. J Child Psychol Psychiatry. 2001 Feb;42(2):241-51. PMID: 11280420.
375. Dodich A, Cerami C, Canessa N, Crespi C, Iannaccone S, Marcone A, Realmuto S, Lettieri G, Perani D, Cappa SF. A novel task assessing intention and emotion attribution: Italian standardization and normative data of the Story-based Empathy Task. Neurol Sci. 2015 Oct;36(10):1907-12. doi: 10.1007/s10072-015-2281-3. Epub 2015 Jun 14. PMID: 26072203.
376. G. Stewart, W.J. McGeown, M.F. Shanks, A. Venneri. Anosognosia for memory impairment in Alzheimer's disease. Acta Neuropsychiatrica, 22 (4) (2010), pp. 180-187, 10.1111/j.1601-5215.2010.00463
377. Starkstein SE, Mayberg HS, Preziosi TJ, Andrezejewski P, Leiguarda R, Robinson RG. Reliability, validity, and clinical correlates of apathy in Parkinson's disease. J Neuropsychiatry Clin Neurosci. 1992 Spring;4(2):134-9
378. Spielberger, C. D. (1999). State-Trait Anger Expression Inventory-2. Lutz, Florida: Psychological Assessment Resources.

ABSTRACT

Myotonic syndromes: analysis of factors with pathogenetic and prognostic significance.

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Background: Myotonic dystrophies (DMs) are progressive autosomal dominant, multisystem diseases with a core pattern of clinical presentation including muscular involvement (skeletal and smooth muscle) with myotonia and dystrophy, cardiac conduction defects, respiratory involvement, ocular disturbances, endocrine and gastrointestinal disorders; moreover, central nervous system involvement (sleep disorder, cognitive impairment, behavioural and psychiatric disorders) is now widely recognized and peripheral nerve involvement has also been described. The natural history of many aspects of DM1 pathology is largely unclear, and systematic longitudinal studies applying standardized clinical, neuropsychological and brain imaging protocols on large cohorts of genetically and clinically well characterized participants are lacking.

Objectives: To evaluate in a monocentric prospective study phenotypic and pathogenetic aspects of myotonic syndromes through the multidimensional and standardized evaluation of cardiological, respiratory and neurological aspects (including both the central nervous system and the peripheral nervous system). More in detail, the main objectives were: to create a computerized database for the collection of clinical and laboratory data; to evaluate sleep disorders (mainly sleep related breathing disorders and excessive daytime sleepiness) through the execution of cardiorespiratory monitoring/polysomnographic examinations; to analyze, through skin biopsies, somatic and autonomic unmyelinated skin fibres and their possible correlation with pain and autonomic symptoms; to study neuropsychological and neuroradiological aspects through MRI.

Methods: A total of 72 DM1 patients (24 E1, 41 E2 and 7 E3) underwent a standardized clinical (neurological, cardiological, respiratory and neuropsychological) and neuroradiological evaluation performed by a multidisciplinary team of dedicated specialists belonging to the same centre that examined patients during 3 years of follow-up. We studied the possible correlations among the different clinical and instrumental parameters, neuroimaging data and genotypic aspects, examining the disease in all its different phenotypic aspects.

Results: We observed that longer disease duration was associated with increased PR length, higher incidence of conduction disorders and lower ejection fraction and we found that higher CVF values were significant predictors for a reduced risk of cardiopathy. In addition, we found that lower functional pulmonary values were associated with class of expansion (showing a significant reduction in E3 patients) and were also negatively associated with disease duration, MIRS and NIFDS score. Higher MIRS values were significant predictors for an increased risk of restrictive syndrome. On the contrary, arterial blood gas parameters did not differ among the 3 genetic classes and were not associated with disease duration nor with respiratory function test or MIRS score. Excessive daytime sleepiness was not associated with class of expansion nor with any of the clinical parameters examined, for the exception of the neuropsychological (NP) domain of the NIFDS scale and COMPASS 31 total score and its subitems. We detected apnoea in a large percentage of DM1 patients, (obstructive but also central apnoea) without significant differences between the 3 genetic classes. We observed that higher CVF values were significant predictors for a reduced risk of apnoea. ODI index and obstructive AHI and mixed apnoea index had significant negative associations with CVF, FEV1 and diurnal pO2, and a positive association with the Daily Life Activity (DL) domain of the NIFDS scale; AHI and mixed apnoea index had also a positive association with PR interval length at ECG. Nocturnal saturation parameters had significant positive correlations with diurnal pulmonary parameters and mean SaO2 showed positive correlation with heart rate. The study of skin biopsy, performed on 45 patients, demonstrated the presence of a subclinical small fibre neuropathy with significant involvement of the somatic fibres but not of the autonomic ones. Males presented a significantly lower fibre density in legs compared to females, while no differences

were found among the 3 genetic classes of patients. The pupillometry study, conducted on 26 DM1 patients, showed a significantly lower pupil size at baseline and a lower constriction response to light. Lower pupil size at baseline was associated with longer disease duration, lower ejection fraction and longer QRS interval length on ECG. Regarding peak amplitude values, we observed that higher constriction response values were associated with a higher percentage of autonomic fibres innervating arrector pili muscles in the thigh on skin biopsy, while lower constriction response values were found in patients with higher disability score and higher apnoea/hypopnea index. Finally, 33 patients of our sample (13 E1 and 20 E2) were studied with a full neuropsychological evaluation and a quantitative structural MRI study. We found that the most affected domains were executive functions and visuoconstructional task, attention, visuospatial, visuoperceptual tasks and visuospatial short-term memory; DM1 patients showed also dysfunction in the social cognition test and presented significant levels of anosognosia, apathy, depression, anxiety and anger. We found a worse performance of E1 patients in the visuoperceptual ability and social cognition tasks, while E2 patients showed a significantly higher global score of apathy and a significant index of mild trait anxiety. All cognitive reserve indices were significantly higher in E1 compared to E2 patients. E1 patients reported a generally higher level of quality of life in all investigated domains. Regarding MRI morphological study, we observed an enlargement of the lateral ventricular volumes and a decrease in the volumes of frontal, parietal, temporal and occipital cortices; at the subcortical level, we found a bilateral decreased volume of accumbens and putamen nuclei. We found a more severe volume reduction of the isthmus cingulate, the transverse temporal, the superior parietal and temporal gyri in E2 patients. On the contrary, we did not find significant differences in white matter lesion load between the 2 genetic classes of patients. We observed a negative association between left lateral ventricle enlargement and performance on short-term verbal memory task, while both left lateral and third ventricles enlargement negatively correlated with emotion attribution abilities in a social cognition task. The volume of right superior temporal sulcus's banks positively correlated with nonverbal reasoning scores. Positive correlations were also found between left lateral occipital volume and performance on visuoperceptive and long-term visuospatial memory tasks. Right middle temporal volume was positively correlated with both visuoconstructional and visuoperceptive abilities, while it negatively correlated with both total score and autonomy in daily activities subitem's scores on the ICS questionnaire. Right superior temporal volume was negatively correlated with executive function abilities. Moreover, we observed that left middle temporal volume was positively correlated with respiratory parameters while a negative correlation was found between left supramarginal volume and central apnoea index. We also found a negative correlation between left and right putamen nuclei volume reduction and O2 saturation levels, and a positive correlation between volume reduction of left putamen nucleus and diurnal pO2.

Discussion: We analyzed a wide spectrum of clinical parameters in a large cohort of DM1 patients, in a monocentric and prospective study that allowed a standardized clinical and instrumental evaluation during 3 years of follow-up. We found some clinical parameters that could predict the risk of cardiopathy, restrictive pulmonary syndrome and sleep related breathing disorder; however other clinical aspects proved to be unpredictable and not related only to the progressive nature of disease nor to the genetic class of expansion, confirming the importance of periodic clinical follow-up of these patients. The natural history of many aspects of DM1 pathology is largely unclear thus systematic longitudinal studies applying standardized clinical, neuropsychological and brain imaging protocols on large cohorts of genetically and clinically well characterized participants are needed. We therefore believe that our study contributed significantly to the phenotypic and genotypic characterization of this pathology and could constitute the basis of a more prolonged follow-up of these patients.