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Die klinischen und diagnostischen Merkmale der Myotonen Dystrophie Typ 2: retrospektive Studie eines großen Patientenkollektivs

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DEUTSCHE ZUSAMMENFASSUNG:

Die Myotone Dystrophie Typ 2 (DM2) ist eine viel jüngere und weniger bekannte Erkrankung im Vergleich zur Myotone Dystrophie Typ 1 (DM1). Aufgrund ihrer niedrigen Prävalenz weltweit resultiert die Beschreibung des DM2 klinischen Bildes seit 1994 hauptsächlich aus klinischen Studien, die mit kleinen Patientenkollektiven (i.d.R. < 50 Patienten) durchgeführt wurden. Ziel dieser Promotionsarbeit ist die Phänotypdarstellung der klinischen Zeichen und Symptome in einer großen Kohorte von DM2 Patienten deutscher Herkunft. Insbesondere sollte der Einfluss von Alter und Geschlecht auf den DM2 Phänotyp erforscht werden.

307 Patienten aus 249 Familien mit einer genetisch gesicherten DM2 wurden in die Studie eingeschlossen. Folgende Daten wurden erhoben: (1) Demographie (Alter, Geschlecht, regionale Herkunft); (2) klinische Zeichen (Symptombeginn, erste Symptome, muskuläre Beschwerden im Verlauf, multisystemische Beteiligung); (3) Diagnostik (serologische Tests, Elektromyographie, Muskelbiopsie). Soweit anwendbar wurden die folgenden statistischen Tests verwendet: Mann–Whitney U-test, Kruskal–Wallis Test, Chi-square oder Fisher's exact Tests. Spezifische Regressionsanalyseverfahren wurden zur Evaluation des Zusammenhangs zwischen unabhängige Variablen (z.B. Alter und Geschlecht) und spezifischen Symptomen durchgeführt.

Die untersuchte Kohorte besteht aus 186 Frauen (61%) und 121 Männer. Bei Erkrankungsbeginn war das führende klinische Leitsymptom eine proximale muskuläre Schwäche (55,4%), gefolgt von Myalgien (35,5%) und der Myotonie (25,4%). Die proximale Muskelschwäche trat häufiger bei Frauen als bei Männern auf (p=0.0006). Hingegen trat bei Männer öfters Myalgien auf (OR=2.94 [95%CI 1.53-5.67]; P = 0.0012). Die Patienten mit Muskelschwäche als Erstsymptom waren älter als solche mit Myalgie und/oder Myotonie (Median 49, vs. 39 und 30 Jahren, p<0.0001). Mit zunehmender Erkrankungsdauer sankt pro Jahr die Wahrscheinlichkeit eine Myotonie zu entwickeln um 10% [OR 0.9 (95% CI 0.87–

0.93) p<0.0001] und Myalgien um 6% [OR 0.94 (95% CI 0.91–0.97), p<0.0001]. Die häufigsten multisystemischen Komorbiditäten waren: Katarakt (49%), Dyslipidämie (41%), Schilddrüsenerkrankungen (32%) und ein Diabetes Mellitus (30%). Katarakt und Schilddrüsenerkrankung traten häufiger bei Frauen (jeweils p = 0,002) als bei Männern auf. Der frühe Erkrankungsbeginn ist ein unabhängiger Risikofaktor für das Auftreten von multisystematischer Organbeteiligung [OR 0.94 (95% CI 0.90–0.98)].

Zusammenfassend konnte in dieser aktualisierten klinischen Phänotyp-Beschreibung der DM2 ein deutlicher Einfluss von Alter und Geschlecht auf den Phänotyp gezeigt werden. Bei Frauen und mit steigendem Lebensalter wird die Krankheitslast progredient größer. Diese alters- und geschlechtsspezifischen Unterschiede müssen bei der Diagnosestellung, beim Management und in zukünftigen klinischen Studien der DM2 berücksichtigt werden.

TABLE OF CONTENTS

LITERATURE REVIEW

1.1 Definition and classification of myotonic dystrophies

Myotonic Dystrophies (DMs) represent the most frequent type of muscular dystrophies in adulthood. Two clinical entities are currently known, the myotonic dystrophy type 1 (DM1, Steinert´s disease) and the myotonic dystrophy type 2 (DM2). They are progressive, autosomal dominant diseases caused by an abnormal expansion of an unstable nucleotide repeat located in the non-coding region of their respective genes (CTG-repeat expansion in DMPK-gene as regards DM1 and CCTG-repeat expansion in the CNBP/ZNF9 gene as regards DM2) *(*Mahadeva *et al.,* 1992; Liquori *et al.,* 2001*)*.

DMs show an extremely heterogeneous phenotype characterized by a combination of muscular (weakness, wasting, myotonia, myalgia) and multisystemic (cataract, heart and endocrine dysfunctions) involvement. Many signs and symptoms of both conditions overlap but important differences allow their clinical discrimination. In DM1 the onset of symptoms ranges from birth to advanced age, whereas DM2 is a disorder of the middle-older age and a congenital form has so far never been described. The clinical course of DM1 is considered to be severer in comparison to DM2, weakness and wasting are distally predominant in DM1 and together with myotonia may greatly affect manual skills *(*Udd *et al.,* 2003*)*. Cognitive impairment, respiratory insufficiency and cardiac complications are more frequent and pronounced in DM1 as in DM2 *(*Meola *et al.,* 2003*;* Sansone *et al.,* 2013, 2015*)* and, most important, life expectancy is reduced in severely affected DM1 patients *(*Mathieu *et al.,* 1999*)*. It has been widely documented that in general a higher number of CTG repeats is associated with a severer phenotype in DM1 *(*Heatwole *et al.,* 2012*)*, whereas this genotype-phenotype correlation has not been observed in DM2 patients *(*Day and Ranum, 2005.*)*. Furthermore, only DM1 patients may show "anticipation" which means that the disease begins earlier and usually with a severer clinical picture, when transmitted from generation to generation, especially by female transmission *(*Duthel *et al.,* 1999*)*.

Having briefly highlighted the most important differences between DM1 and DM2, this work will further focus on the most relevant aspects of DM2.

1.2 Brief history of myotonic dystrophy type 2 (DM2):

In comparison to DM1, described for the first time in 1909 by Steinert, DM2 is quite a young disease as its history begins about 20 years ago. It was at the very beginning of the 1994 that Thornton et al. in the USA described 3 patients presenting clinical features suggestive of a myotonic dystrophy who, however, carried no CTG-repeat expansion *(*Thornton *et al.,* 1994*)*. In this first description, the authors already pointed out some important peculiarities that differentiated these patients from the typical DM1 picture, in particular the predominantly proximal muscle weakness and the lack of anticipation. Few months later that year, Ricker et al, in Germany, described 3 families (15 patients) presenting various combinations of proximal muscle weakness, myotonia and cataract, likewise showing no mutations in *DM1*, *SCN4A* or *CLCN1*. They proposed the term "Proximal myotonic myopathy – PROMM" for this newly discovered clinical entity *(*Ricker *et al.,* 1994*)*.

The first insights towards its genetic characterization came from the group of John Day and Laura Ranum. In 1998 they could map, in a 5-generation family, the disease locus on chromosome 3 *(*Ranum *et al.,* 1998*)*. Since some of these patients presented a distal instead of a proximal muscle weakness, the authors proposed the name "myotonic dystrophy type 2 – DM2".

In 1999 Ricker et al. confirmed that also in their PROMM patients the responsible gene was located in the same region of chromosome 3 (Ricker *et al.,* 1999). Consequently, it was for some years considered the possibility that PROMM and DM2 were either diseases caused by two closely linked genes or two allelic disorders; the first, characterized by a predominantly proximal muscle weakness and the latter by distal muscle weakness.

It was finally in 2001 that Liquori et al. identified, in the Minnesota DM2 family and in several German PROMM patients and families, the causing gene (zinc finger protein 9 gene - *ZNF9/CNBP*), its location (3q21) and demonstrated that a CCTG-repeat expansion in the intron 1 of the *ZNF9/CNBP* was involved in the disease process *(*Liquori et al., 2001*)*. In 2003, the CCTG-expansion mutation was also confirmed in PROMM patients of other non-US-German origin like Italy and Finland *(*Bachinski *et al.,* 2003*)*. Hence, the confusion related to the several labels and eponyms proposed in the past ("myotonic dystrophy with no trinucleotide repeat expansion", "Mox-pox syndrome", "Thornton-Griggs-Moxley disease", "proximal myotonic myopathy – PROMM",

"myotonic dystrophy type 2 – DM2", "proximal myotonic dystrophy - PMD", Ricker disease) could be solved. Finally at an ENMC workshop on Myotonic Dystrophy in 2003, it was proposed that all types of DM2 and PROMM refer to the same condition and should be termed as "myotonic dystrophy type 2 – DM2" (Udd *et al.,* 2003).

1.3 Epidemiology

The prevalence of DMs, based on clinical diagnosis, has been estimated at 12.5:100.000 (Harper, 2001*)*. DM1 prevalence varies between 0.43 and 178 per 100.000 in different populations *(*Vanacore *et al.,* 2016*),* whereas the precise incidence/prevalence of DM2 has not been specifically studied yet. The large majority of DM2 patients are Europeans with the exception of few families of North Africa, Afghanistan and Sri-Lanka *(*Udd *et al.,* 2003*)*. In 2003 Bachinski et al discovered that DM2 patients of different origin share a common haplotype and hypothesized that a single founder mutation could be responsible for DM2 origin (Bachinski *et al.,* 2003; Coenen *et al.,* 2011). Being DM2 virtually absent in east-Asia *(*Matsuura *et al.,* 2012*)* and Sub-saharan populations, it has been postulated that this ancestral mutation might have occurred about 35.000 years ago after the "out-of-Africa" migration, when the divergence between European and Asian lineages started *(*Bachinski *et al.,* 2003). This hypothesis finds its confirmation in the first Asian patient reported who presented a haplotype distinct from the European one suggesting a separate founder effect *(*Saito *et al.,* 2008*)*. A family of apparent Afghan/Tajik ancestry, was shown to share the common European haplotype, suggesting that the DM2 expansion occurred prior to the Aryan migration of Indo-Europeans that settled Aryana (ancient Afghanistan) in 2000–1000 BC *(*Schoser *et al.,* 2004b*)*.

If a common founder mutation is then responsible for European DM2 cases, one would expect the disease prevalence to be similar across Europe, nevertheless major differences within countries have been reported. In fact, in some nations as Finland, Germany and Czech Republic the DM2 frequency is much higher than in other regions, reaching a similar prevalence, or even higher to that of DM1, up to 1:1830 *(*Udd *et al.,* 2011; Suominem *et al.,* 2011*)*. On the other hand, other countries as Italy have an estimated DM2 prevalence of 1:100.000, about 10% that of DM1 *(*Vanacore *et al.,* 2016*)*.

1.4 Genetic aspects and pathogenesis

DM2 is caused by an abnormal expansion of a tetranucleotide CCTG-repeat in the first intron of the *CNBP/ZNF9* (3q21). Non-pathogenic alleles contain a complex repeat structure of $(TG)_{14-25}(TCTG)_{4-10}(CCTG)_{11-26,}$ where the last tetranucleotide might present up to 26 repeat units often interrupted by other tetranucleotide repeats (GCTG/TCTG, TCTG/TCTA). In affected alleles the CCTG-expansion ranges between 75 and 11.000 uninterrupted repeats *(*Liquori *et al.,* 2003*)*. The CCTG-expansion is extremely unstable as it increases with aging and shows a marked heterogeneity within different tissues of the same subject or even among different blood samples (Udd *et al.,* 2003*)*. Differently from DM1, no correlation and anticipation has been found between the size of the CCTG expansion and phenotype severity in DM2 patients.

DM2 **pathogenesis** remains elusive and many hypotheses have been translated from studies on DM1. Two major mechanisms are nowadays considered: 1) a possibly reduced expression of *CNBP* (*CNBP* haploinsufficiency); and more likely 2) RNA toxicity with secondary disruption of the transcription/splicing of many genes *(*Mateos-Aierdi *et al.*, 2015). As regards the first mechanism, the real contribution of CNBP deficiency is still unclear; on one hand *CNBP/ZNF9*-knock out mice developed many of the multisystemic features of DM2 *(*Chen *et al.*, 2007*)*, but on the other hand some studies have instead found that the CCTG-mutation did not determine a reduction in CNBP protein levels *(*Margolis *et al.,* 2006*)*. Nevertheless, other studies showed a clear reduction of CNBP protein *(*Schoser and Timchenko, 2010*)*. Moreover, the decrease of proteins of translational apparatus in DM2 correlates with a reduced rate of protein synthesis in myoblasts from DM2 patients and the ectopic expression of CNBP in DM2 myoblasts corrects the rate of protein synthesis. This suggests that the alterations in CCUG-ZNF9-TOP mRNAs pathway might be responsible for the reduction of the rate of protein translation in DM2 muscle cells (Huichalaf *et al.,* 2009*).*

A larger number of studies have investigated the mechanisms of RNA toxicity and its role in impaired transcription/splicing. The abnormally expanded CCTG-repeat is transcripted in abnormally expanded $(CCUG)_n$-RNAs that accumulate in cell nuclei ("ribonuclear inclusions", "RNAs foci"). These inclusions interact and influence the function of at least two families of RNA-binding proteins:

the muscleblind-like proteins (MBLN1,2,3) and the CUGBP/CELF family. While in DM1 both the sequestration of MBLN1 and the overexpression of CUGBP1 are equally important for the pathogenesis, in DM2 models the role of CUGBP1 is less clearly defined and its overexpression has been inconstantly found so that the sequestration/inactivation of MBLN1 seem to be the major determinant in DM2 pathogenesis *(*Schoser and Timchenko, 2010; Lukas et al., 2012; Cardani et al., 2013*)*.

MBLN1 regulates the splicing of several target genes whose aberrant proteins are responsible for the multiform and multisystemic clinical features in DM2. For example, the aberrant splicing (AS) of the insulin receptor explains the increased occurrence of insulin resistance and/or overt diabetes *(*Savkur *et al.,* 2004*)*, the AS of the skeletal muscle chloride channel (CLC-1) is one of the determinants of myotonia (Choi *et al.,* 2015), the accumulation of specific tau-isoforms (AS of the microtubule-associated tau protein - MAPT) in DM2 brain might explain some of the structural and functional changes observed in patients (white matter changes, grey matter atrophy, cognitive dysfunctions) (Caillet-Boudin *et al.,* 2014*)*, AS of the cTNT (*TNNT2*) coding for cardiac troponin might be involved in heart dysfunction *(*Vihola *et al.,* 2010*)*. Other genes aberrantly expressed in DM1 and in DM2 are e.g. RYR1, SERCA1 and Cav1.1 that may alter the intracellular Ca2+ signalling and sarcolemmal excitability and still an increasing number of genes aberrantly spliced in DMs emerge from genome-wide studies *(*Vihola *et al.,* 2010; Perfetti *et al.,* 2014*)*.

1.5 DM2 clinical features

DM2 is usually considered as a benign condition in comparison to DM1, the onset of symptoms occurs later, usually in the 3rd-5th decade, and the clinical course is generally mild and slow. Nevertheless, severe cardiac complications and/or progressive muscle weakness leading to wheelchair use have been reported *(*Udd *et al.,* 2003, 2011*)*. DM2 clinical picture is characterized by a combination of muscular and multi-systemic signs and/or symptoms. The following summary is ordered by the clinical frequency of DM2 symptoms.

1.5.1 Muscle Involvement

Muscular symptoms are the most frequently reported complaints and often represent the reason for referral to doctors. The largest cohort of DM2 patients so far clinically described accounts of 234 subjects, who presented as most frequent muscular complaints: muscle weakness (82%), clinical myotonia (75%) and muscle pain (myalgia) (56%), *(*Day *et al.*, 2003*)*.

The **proximal muscle weakness** was the characterizing feature in the first description of "PROMM" patients *(*Ricker *et al.*, 1994*)* and the muscle groups earlier and more consistently involved were the neck flexors, thumb and deep-finger flexors, hip flexors and extensors *(*Day *et al.*, 2003*)*. The pattern of muscle involvement has also been studied with MRI that showed the early degeneration of the erector spinae and gluteus maximus muscles, thus confirming the mainly axial/proximal weakness distribution *(*Kornblum *et al.*, 2006*)* (Fig. 1). The severity of muscle weakness is usually mild to moderate and only a minority of patients (~10%) will then require a wheelchair in the course of the disease *(*Udd *et al.*, 2011*)*.

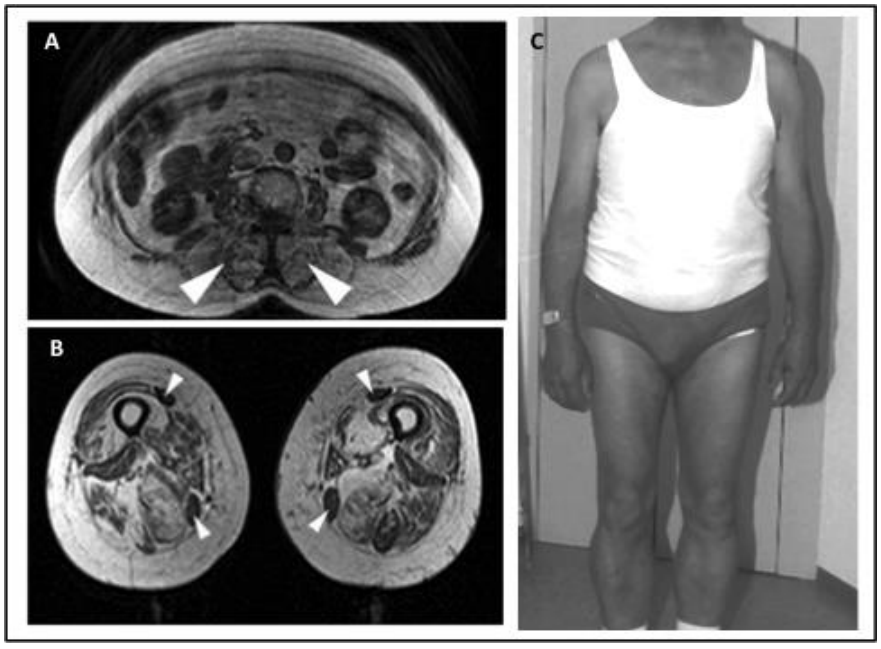

Fig. 1 Proximal muscle weakness in DM2. A,B: DM2 patient (MRI T1weighted images) **A)** symmetric severe fatty degeneration of the erector spinae muscles (arrow heads); **B)** fatty degeneration of the vastus medialis, intermedius and lateralis as well as the long head of biceps femoris, semimembranosus and adductor muscles *(Kornblum et al 2006)*; **C)** Mild atrophy of thighs muscles with relative calf hypertrophy

Myotonia is defined as a delayed relaxation of skeletal muscles after voluntary contraction, and is usually mild or even absent in many DM2 patients, impacting only minimally their quality of

life *(*Heatwole *et al.,* 2015*)*. Its occurrence, in different cohorts, ranges between 24% and 75% (Day *et al.*, 2003; Young *et al.,* 2010*)*. This variability is partly due to the discrepancy sometimes observed between myotonia anamnestically reported by patients and the clinical evidence of myotonic phenomenon observed during the neurological examination. A minority of patients may display a severe myotonia and in some of these patients additional mutations in genes regulating ion channels function (CLCN1, SCN4A) have been identified (Cardani *et al.,* 2012; Bugiardini *et al.* 2015).

Already in the first descriptions of DM2, a "peculiar **pain**" was reported from the majority of patients (Ricker *et al.,* 1995*).* Its occurrence varies in different studies between 50%-95% of cases (Ricker *et al.,* 1995; Ricker *et al.,* 1999; George *et al.,* 2004). Pain is usually described as: exercise-related, cold- and palpation-induced, quite variable as regards the sites involved (spine, proximal and distal muscles), often with a radiating tendency, lasting for hours or days (George *et al.,* 2004). It is not exclusively a muscular pain, it might be in some cases widespread involving also joints and sharing some features with fibromyalgia (Auvinen *et al.,* 2008), or it might be characterized by recurrent headaches and/or abdominal pain (Tielemann *et al.,* 2008; Suokas *et al.,* 2012). Some patients consider pain as the most disabling symptom as it also has a not satisfactory response to common analgesics (Udd *et al.*, 2006).

Less commonly reported muscular complaints include cramps, fasciculations and mild dysphagia (Udd *et al.,* 2011); a calf hypertrophy is also present in a subgroup of DM2 patients (Thornton *et al.*, 1994).

1.5.2 Multisystem involvement

Beside the muscular involvement, DM2 patients experience a progressive dysfunction of several organs and systems that become more frequently and more severely affected with aging, thus resembling a progeroid syndrome (Mateos-Aierdi *et al.*, 2015). The most frequently occurring multi-systemic complications are: cataract, cardiac complications (arrhythmias, cardiomyopathy) and endocrine dysfunctions (e.g. diabetes, thyroid diseases and hypogonadism). Less often, gastro-intestinal symptoms (constipation, diarrhea) (Tieleman *et al.,* 2009), hearing loss (Thorton *et al.*, 1994), autoimmune diseases (Tieleman *et al.,* 2008), central nervous system affections (Meola *et al.*,

2003; Schneider-Gold *et al.,* 2015) and a higher incidence of tumours (Gadalla *et al.,* 2011, 2013, 2016) have also been reported in some cohorts. The reason why some tissues are affected more severely than others is still unclear, but it may rely on the distribution and expression of the modifying splicing factors and the propensity of some cell types to extend the CCTG-repeat in their cell cycle, so that different tissues bear different repeat lengths configuring a somatic mosaicism.

About 60% of DM2 patients present a posterior subcapsular iridescent **cataract** on slit lamp examination, occurring at a mean age of 45 years, sometimes even as first sign of the disease (Day *et al.*, 2003). **Cardiac** abnormalities in DM2 (sudden cardiac death, atrio-ventricular conduction defects, cardiomyopathy) (Schoser *et al.,* 2004a; Wahbi *et al.,* 2009; Sansone *et al.,* 2013) are similar to those observed in DM1 but occur less frequently. According to a recent observational case-control study on a large cohort of DM2/DM1 patients, it emerged that electrocardiographic abnormalities as PR>200ms and QRS>100ms were more frequent in DM1 (respectively 31% and 48%) than DM2 patients (10% and 17%). Of those, 6 DM2 vs. 28 DM1 patients needed a pacemaker/implanted cardioverter (Sansone *et al.*, 2013). In the same study, echocardiography did not show any significant structural abnormalities but it was previously reported that a cardiomyopathy might occur in about 3% of DM2 patients (Day *et al.,* 2003; Schoser *et al.,* 2004a; Sansone *et al.,* 2013).

Unlike DM1, the **endocrine function** of DM2 patients has not been systematically investigated but several studies report an increased incidence of insulin resistance/glucose intolerance (23%), primary hypogonadism (20-46%), thyroid dysfunction and hyperparathyroidism (Day *et al.,* 2003; Savkur *et al.,* 2004; Passeri *et al.,* 2015). Regularly monitoring the occurrence of these comorbidities is particularly important as they may worsen the muscular symptoms (Sansone *et al.,* 2000) and favour cardiovascular complications.

A relevant **respiratory impairment** rarely occurs in DM2 and only about 6-15% of patients require non-invasive ventilation (NIV) (Sansone and Gagnon, 2015). The major causes of respiratory involvement are a restrictive respiratory insufficiency together with diaphragmatic weakness and sleep apneas (Leonardis *et al.,* 2014).

In the past decade several authors have studied the **central nervous system** involvement in DMs. In comparison to healthy controls, mild cognitive and behavioural symptoms were detected in

DM2 patients, mainly characterized by altered visuo-spatial and executive functions, reduced attention and flexibility of thinking, avoidant behavioural trait and depression (Meola 2003, Schneider-Gold 2015). These observations partly correlate to alterations detected in functional and structural neuroimaging studies (Meola *et al.,* 2003; Minnerop *et al.,* 2011; Schneider-Gold *et al.,* 2015). SPECT showed a reduced blood flow in the frontal and parieto-occipital regions (Meola *et al.,* 2003) and voxel-based morphometry documented grey and white matter atrophy with different distribution in DM1 and DM2 patients. This latter group showed more WM reduction and atrophy of the limbic and brainstem structures in comparison to DM1 patients who had major GM atrophy with affection of the central motor pathways (Minnerop *et al.,* 2011; Schneider-Gold *et al.,* 2015). Cerebral white matter hyperintensities have been observed in both DM1 and DM2 patients, especially if older than 40 years, but their clinical and functional significance still remains unclear (Franc *et al.,* 2012; Schneider-Gold *et al.,* 2015; Kornblum *et al.,* 2004).

An increased risk for **neoplasm** has been associated to DM1/2 and cancer represents the third cause of death in DMs (Gadalla *et al.,* 2013). Patients seem to have twice the risk of developing tumours in comparison to the general population and also in comparison to their not DM-affected relatives (Lund *et al.,* 2014). As expected, the absolute risk increases with age (over 40 years) and seems slightly higher in females (Gadalla *et al.,* 2013). The most frequent cancers affect the endometrium, brain (astrocytomas), ovary, thyroid and colon (Gadalla *et al.,* 2011, 2016). Other more benign tumours frequently reported in DM1 are the pilomatricoma, a calcinous neoplasm of the skin, and the basal cell carcinoma (Zampetti *et al.,* 2015).

1.6 Diagnosis

Considering its wide clinical spectrum, the early diagnosis of DM2 still represents a challenge for clinicians. Mild and very late-onset forms may remain undiagnosed as many signs and symptoms overlap with normal aging, thus going unrecognized or being attributed to other diseases (Hilbert *et al.,* 2013). Furthermore, the phenotype is often incomplete and some core features like positive family history, cataract, endocrine dysfunction, clinical myotonia or myotonic discharges on EMG may be absent in a large number of patients at the beginning of symptoms (Toth *et al.,* 2007; Milone *et al.,*

2009). For these reasons DM2 patients often undergo, before reaching the diagnosis, more diagnostic exams (EMG, muscle biopsies) in comparison to DM1 patients and the average diagnostic delay is twice as long (DM2 mean 14 ±12.8 years vs. DM1 7.3 ±8.2 years) (Hilbert *et al.,* 2013).

Besides family history and clinical signs/symptoms, the most valuable hints towards DM2 diagnosis can be obtained from serologic assessments, electromyography and, possibly, muscle biopsy. Finally, the detection of the abnormally expanded CCTG-repeat (>75 CCTG-repeat units) of the *CNBP* will confirm the diagnosis.

1.6.1 Serologic assessments:

Many laboratory parameters are often altered in DMs, sometimes revealing multi-systemic complications (thyroid diseases, diabetes mellitus, hypogonadism, etc.). Unlike DM1, only few studies have specifically investigated the laboratory profile of DM2 patients (Day *et al.,* 2003; Heatwole *et al.,* 2011).

The most frequently abnormal laboratory parameters are: CK (elevated in 83%), IgG (reduced in 75%), total cholesterol (elevated in 63%), glucose (elevated in 43%), alanine aminotransferase (ALT) (elevated in 50%), lactate dehydrogenase (LDH) (elevated in 50%), total protein (reduced in 43%) and gamma-glutamyltransferase (GGT) (elevated in 33%) (Heatwole *et al.,* 2011).

1.6.2 Electromyography:

Neurophysiological studies play an important role in the diagnostic approach, as the detection of myotonic discharges on EMG noticeably narrows the differential diagnosis. Besides dystrophic (DMs) and non-dystrophic myotonias (NDMs), myotonic discharges (MDs) can be rarely found in other neuromuscular diseases as glycogen storage disease type 2 (GSD2), myofibrillar and centronuclear myopathies and sporadic inclusion body myositis (sIBM) (Hanisch *et al.,* 2013, 2014). On the other hand, the absence of MDs on EMG is not a sufficient criterion to exclude DM2 as 10-25% of patients may present at the time of diagnosis no electrical myotonia or atypical myotonic discharges (Young *et al.,* 2010).

According to the type, distribution and triggers of myotonic discharges several studies have tried to differentiate between DMs and NDMs. In 2004, Fournier et al. applied short and long exercise

tests in myotonic syndromes describing different EMG patterns (changes in CMAP amplitude) in myotonia congenita, paramyotonia and periodic paralysis thus guiding the subsequent genetic analysis according to the type of channelopathy (Fournier *et al.,* 2004). Even if the differential diagnosis between DM1 and DM2 is mainly clinical, some additional differences detected on EMG can also be taken into account. Overall, myotonic discharges are found significantly more often in DM1 patients, being detected in almost all muscle groups, the exception is represented by vastus lateralis and tensor fascia lata muscles where MDs are mainly detected in DM2 patients (Logigian *et al.,* 2007). In both DM1 and DM2 distal muscles display more commonly MDs in comparison to proximal ones. Furthermore, the classical myotonic discharges, with a waxing and waning trend, are way more frequent in DM1 than DM2 patients, who instead show many atypical or incomplete myotonic discharges presenting only the waning phase or show other forms of pathological spontaneous activity as jasper or complex repetitive discharges (Logigian *et al.,* 2007; Young *et al.,* 2010). Also the application of the short exercise test and the short exercise test with cooling may help differentiating the two DMs, as in DM1 differently from DM2 a reduction of cMAP amplitude after effort is observed (Gawel *et al.,* 2013). It seems then that in DM2 patients MDs are more frequently found in proximal muscles (e.g. ileopsoas and paravertebral muscles), which are commonly not investigated in a routine setting (Schoser personal communication).

1.6.3 Muscle Biopsy:

Differently from DM1, where muscle biopsy is very rarely performed during the diagnostic ascertainment, in DM2 still about 40% of patients undergo a muscle biopsy before the proper diagnosis is reached (Hilbert *et al.,* 2013). This is particularly true in those countries where the prevalence of DM2 is quite low or genetic testing unavailable.

The typical histopathological picture is characterized by nuclear changes as increased number of centralized nuclei (>5) with pyknotic nuclear clumps, small, angulated fibres and increased fibre calibre variability with a predominance of atrophied type 2 fibres (denervation-like pattern) (Schoser *et al.,* 2004c). Even if these alterations might be considered quite unspecific, taken individually, the concomitant occurrence of type 2 fibres atrophy and central nucleation (selectively affecting type 2 fibres) was the most predictive histological feature of DM2 in a large series of muscle biopsies

(Bassez *et al.*, 2008). For these reasons, DM2 is considered a "disease of type 2 fibres" as the majority of alterations is observed here. These histological changes are consistently observed independently from the biopsied muscle and from clinical picture; even if in patients with a longer disease course they become more obvious.

1.6.4 Genetic analysis:

Eventually, the diagnostic confirmation should come from genetic studies. However, the uniquely large size (>40Kb) of the CCTG-repeat expansion has in the past complicated the molecular diagnosis since the direct Southern blotting analysis, formerly adopted, was vitiated by a low sensitivity (80%). In the last years, new molecular approaches have been developed and validated, having nowadays the genetic analysis a specificity and sensitivity of >99%. The currently used recommendations for the genetic diagnosis suggest a step-wise approach encompassing: 1) conventional PCR and fragment length analysis to assess whether an individual has 2 normal-sized alleles 2) if only 1 normal allele is detected, a quadruplet-repeat primed PCR (QP)-PCR and/or southern blotting of long-range PCR products are then used to confirm the presence of the expansion (Kamsteeg *et al.*, 2012). Due to the proved lack of correlation between expansion-size and phenotype and to the extreme variability of CCTG-repeats in different samples from the same subjects it is not considered anymore necessary the precise quantification of the expansion and many laboratories do not report its exact length (Kamsteeg *et al.*, 2012).

1.7 Treatment

To date, no disease-specific treatment exists for both DMs and almost all experimental studies currently ongoing are performed on DM1 cellular and animal models, with the hope that the effective ones could be then translated also to DM2. This situation is also referred to the non-existence of an ideal DM2 mice model. However, the increasing knowledge on the pathogenic mechanisms, especially the splicing alterations and RNA toxicity involved in DMs has led to the identification of new potential therapeutic targets. The most promising approaches include the use of antisense therapies and

the regulation of downstream disease mediators such as MBNL1 and CELF1molecules. Antisense nucleotides acts inhibiting the interactions of the toxic RNA and nuclear proteins (MLBN1, CELF) (Gao and Cooper, 2013; Kiliszek *et al.,* 2016; Pandey *et al.,* 2015; Wojtkowiak-Szlachcic *et al.,* 2015), promoting targeted degradation of repeat expansion mRNA, and reducing the size of the trinucleotide expansion (Leung *et al.,* 2013). Other approaches include, among others: the overexpression of MLBN1 ameliorating the aberrant splicing of different target genes (Chen *et al.,* 2016), studies with small molecules inhibiting the interaction between triplet-expansions and MBNL1 (Nakamori *et al.,* 2015), RNA interference therapies adopting adenoviruses vectors (Bisset *et al.,* 2015) and DMPK downregulation (Witherspoon *et al.,* 2015).

Beyond experimental studies, therapeutic efforts are oriented towards the improvement of muscular symptoms and the prevention/management of complications and comorbidities of DM2. To improve myalgia several drugs might be adopted, including NSAIDs, anti-epileptic drugs (gabapentin, pregabalin), central-acting muscle relaxants (methocarbamol) and several antidepressants (amytriptilin, mirtazapine, citalopram); nevertheless no specific medication has shown consistent benefit (George *et al.,* 2004; Udd *et al.,* 2006). Similarly, for myotonia and stiffness the same drugs adopted in other diseases are prescribed (Flecainid bis 2 x 100 mg/d, Propafenon bis 2 x 300 mg/d, Carbamazepin bis 3 x 200 mg/d, Lamotrigin, Gabapentin) (Udd *et al.,* 2011). Furthermore, some guidelines have been developed as regards the management and prevention of respiratory involvement, cardiac complications and endocrine dysfunctions (Elliot, 2014; Sansone *et al.,* 2012, 2015).

2. STUDY BACKGROUNDS AND AIMS:

As described above, DM2 is a far younger and less well studied disease in comparison to DM1. The majority of studies offering a detailed clinical description are quite old, often antecedent to the discovery of the responsible genetic mutation and mainly performed on small groups of patients. On the other hand, the study by Day et al reported the clinical features of a subgroup of patients (n=234) where the known core features of DM2 were discussed, however the main focus of that study was the genetic confirmation in a large group of DM2 patients.

Many studies have then investigated the multisystemic involvement in DM2 but only for the most well-known comorbidities (diabetes, cataract and cardiac involvement) there is undoubted evidence for a higher prevalence in DM2. In regards to other comorbidities (gastro-intestinal symptoms, hearing loss, autoimmune diseases, tumours, etc.) the difficulties in recruiting large number of patients did not permit to draw conclusive statements and further researches are needed to clarify whether these multi-systemic affections occur with higher frequency in comparison to the general population. In DM1 it has also been recently documented that gender influences some disease manifestations which might occur with different frequency between males and females *(Dogan 2016)*; the impact of gender as modifying factor has instead never been studied in DM2.

Some authors have then tried to delineate the laboratory profile of DM2 patients. This has been specifically studied only in two papers, the first one (Day *et al.*, 2003) investigated a large number of patients but few laboratory parameters (CK and GGT were assessed in 150 patients, and IgG, IgM, IgA, FSH, testosterone in about 20 patients); the second study, on the other hand, analysed a large dataset of laboratory parameters (~60) but on a small cohort of DM2 patients (n=49) (Heatwole *et al.,* 2011). These assessments, moreover, did not evaluate the presence of comorbidities or the intake of medications (statins, insulin) at the time of laboratory sampling and no correlation was performed as regards patient´s age or gender.

In the light of these observations, the main areas of interests of this work were: 1) provide a clinical description of the so far largest group of genetically confirmed DM2 patients and assess how aging and gender influence the phenotype; 2) consider which systems and organs are more consistently and earlier affected in the disease course; 3) evaluate the serologic profile of DM2 patients; 4) collecting additional information regarding the geographical origin of our DM2 patients and their ancestry to identify whether some geographical regions show an higher prevalence of DM2; 5) identify disease hallmarks that orient toward an earlier DM2 diagnosis to overcome the huge gap in diagnostic delay.

3. METHODS

3.1 Patients

Two databases were used in order to identify patients with DM2: 1) the German-Swiss Registry for Myotonic Dystrophy (DM-Register) (https://www.dm-registry.org/de/); 2) the internal database of the Friedrich-Baur-Institute (FBI), Department of Neurology, Ludwig-Maximilian University, Munich, Germany.

Patients were considered suitable for this study if they had: a) a genetically confirmed diagnosis of DM2; b) a minimal data-set of clinical information in at least one database including: age at onset, age at diagnosis, symptoms at onset and at the time of last follow-up, comorbidities. Written informed consent to be included in the databases was obtained from all patients. The study was approved by the local ethic committee (document n°107-01,292-07,477-13).

3.2 Data collection:

Clinical data were collected retrospectively analysing: 1) medical chart records (from 2001 to 2016), 2) data entries provided in the patients´ registry, 3) postal surveys.

Two questionnaires have been specifically designed and sent per post or email to patients:

- ***Questionnaire n°1***: it consisted of simple questions aiming to assess where the ancestors of DM2 patients came from. We have asked patients to indicate the place of birth (city, region, state) of their parents and grandparents, indicating who of them most probably had/transmitted the disease. This survey was sent to those patients who furnished their email address (n= 256). *(Attachment n°1, Supplementary material).*
- ***Questionnaire n°2:*** encompassed questions on general clinical information including, among others, age at onset, first symptom, age at diagnosis, multi-system involvement, actual and past medications. This survey was sent per post to 312 patients. *(Attachment n°2, Supplementary material).*

Eventually, the following data could be recorded and tabulated:

a) Demographics (age, gender, family history and origin);

b) Clinical data (age at onset, first symptom, age at diagnosis, diagnostic delay, neurological examination, presence and distribution of myotonia, muscle weakness and pain, multi-systemic involvement);
c) Diagnostic assessments (serological tests at diagnosis, electromyography/electroneurography performed at the time of diagnosis, muscle biopsy, genetic analysis);
d) Follow-up

The family history was considered "positive" if patients reported of family members diagnosed with DM2 or complaining of suggestive symptoms. As "disease onset" we have considered the onset of muscular symptoms. The "diagnostic delay" was defined as the time interval between disease onset and diagnosis.

At least one neurological examination was performed in DM2 patients of the Friedrich-Baur-Institute database. Even if not fully standardized, it included a manual muscle testing (Medical Research Council Scale) and search for myotonia (action- and percussion-induced myotonia).

The presence of comorbidities (cataract, dyslipidaemia, hypertension, thyroid dysfunction, diabetes mellitus, affective disorders, heart diseases, respiratory impairment, hearing loss, asthma, tumours, skin changes, daytime sleepiness, gallstones, stroke) was assessed reviewing the medical records and the serological tests performed. The following laboratory parameters were considered: CK, ALT, AST, GGT, LDH, glucose, HbA1C, IgG, IgA, IgM, total cholesterol, LDL-cholesterol, HDL-cholesterol, triglycerides, vitamin D, PTH, TSH, free T3, free T4, testosterone. Normal values were defined according to the standardized references of the laboratory of the Ludwig-Maximilian University. Only laboratory studies performed within 2 years from the diagnosis where considered. For patients that, in this specific time interval, underwent more than one laboratory assessment, the mean value was calculated and then used for the final analysis. Not every patient underwent all the serologic assessments. The presence of comorbidities (history of hypercholesterolemia, diabetes, thyroid dysfunction) and the intake of medications (lipid lowering drugs, anti-diabetics, substitutive hormones) influencing some laboratory results, where also considered for the analysis.

3.3 Statistic analysis:

Statistical analysis was performed using IBM SPSS statistics 23.0 software. Exploratory analysis was performed to determine the distribution of the variables. Comparisons were made using the Mann–Whitney U-test (two groups) or the Kruskal–Wallis test (three or more groups). Chi-square or Fisher's exact tests were used to compare categorical variables across patient subgroups. The relation between biological parameters was assessed by bivariate correlations (Spearman's rho).

A multinomial regression analysis was performed to evaluate the association between the independent variables (age and sex) and the type of symptom or comorbidity at onset as the dependent nominal outcome. Logistic regression was used to analyse the association between the systemic involvement (dependent variable) and patient characteristics (independent variables). All statistical tests were performed two-sided and a p-value <0.05 was considered significant.

4. RESULTS:

4.1 Patients

A total of 437 patients were identified with a diagnosis of Myotonic Dystrophy type 2. Only those patients with a confirmed molecular diagnosis and sufficient clinical details were included in this study. The final number of patients studied was 307 from 249 families.

Our cohort is composed of 186 females (60%) and 121 males (40%) aged between 21 and 90 years-old with a mean age of 58 years (±13.4). No significant age difference was present between males (mean age 57±13.4) and females (mean age 59±13.3) (p=0,2). A positive family history was referred from 66% of our patients (n= 202) (Tab. 1).

Demographics	
DM2 patients, age	n=307, 58yrs (±13.4)
DM2 families	n=249
Females/males	F/M=1,5
Females, mean age	n=186 (60%), 59yrs (±13.3)
Males, mean age	n=121 (40%), 57yrs (±13.4)
Positive family history	n=202 (66%)

Tab.1: DM2 patients' demographics

4.2 Epidemiological features:

Most patients were Caucasians, of European descent, being the large majority of them Germans; only few families came from Afghanistan, Greece, Austria, Poland and Czech Republic.

Among those patients who received the survey regarding the origin of their family (n=256), 130 patients (130 families), answered the questionnaire (response rate 51%). All patients could report the birthplace of their parents and 106 patients could provide sufficiently complete information on their grandparents´ birthplace. In Table 2 the origins of DM2-ancestors are summarized. Comparing the origin of the affected vs. not affected parent it has interestingly emerged that a high proportion of the DM2 affected parents (37% vs. 16%, p=0,004) had Polish origin, being the Upper/Lower Silesia region the most represented (85%) (Fig. 2).

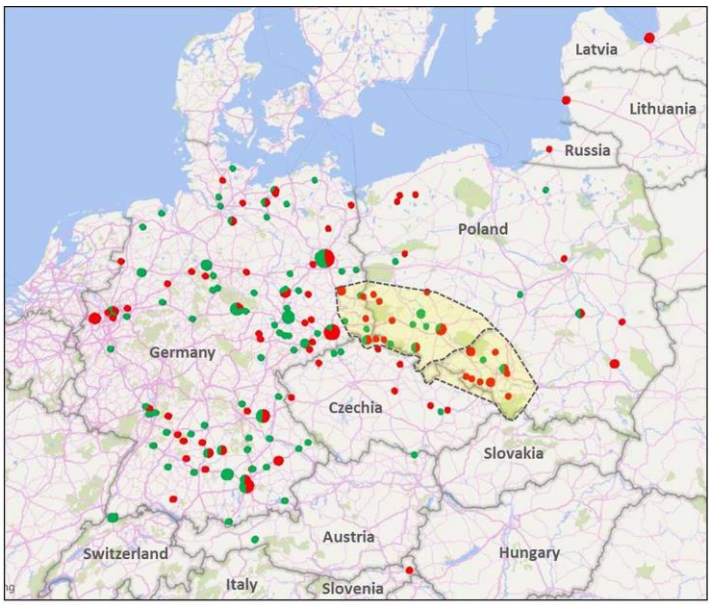

Fig. 2 Origin of DM2 patient's ancestors Parents of DM2 patients (n=236). Yellow area= upper and lower Silesia. Red= affected parents; green= not affected.

Generation (patients cohort)	DE	POL	East Europe	No data	TOT	p-value
DM2 patients	120	8	2	0	130	-
II Generation (patients parents)	**DE**	**POL**	**East Europe**	**No data**	**TOT**	**p-value**
Not affected	93 (79%)	19 (16%)	6 (5%)	12 (9%)	130	
presumably DM2	62 (52%)	44 (37%)	12 (10%)	12 (9%)	130	**0.000415**
TOT	155	63	18	24	260	
III Generation (patients grandparents)	**DE**	**POL**	**East Europe**	**No data**	**TOT**	**p-value**
Not affected	106 (76%)	26 (19%)	8 (6%)	93 (40%)	233	
One possible	86 (44%)	86 (44%)	24 (12%)	37 (16%)	233	**0.00001**
TOT	192	112	32	130	466	

Tab.2 Origin of DM2 patients ancestors

4.3 Clinical features:

A summary of the clinical characteristics of this cohort is reported in Table 3.

DM2 clinical features		Present study	Day et al 2003[6]	Hilbert et al 2013[10]
Numbers of patients (families)		n=307 (249)	n=234/379 (133)	n=135 (n.a.)
F/M		186 (61%)/121 (39%)	210/169	n.a.
Age (mean±SD)		58 (±13.4)	47 (±?)	n.a.
Age at onset (mean±SD)*		42 (±13.8)	37(±15)	34 (±14.1)
Age at diagnosis (mean±SD)		50 (±12.6)	47	48 (±12.2)
Diagnostic delay		5 (0-35) (IQR 1-12)	n.a.	14 (±12.8)
Initial symptom*		Weakness 55% Myalgia 35% Myotonia 25% Cataract 14%	Myotonia 40% Weakness 39% Pain 16% Cataract 8%	Weakness 54% Myotonia 31% Pain 16% Cataract 5%
Symptoms at last clinical follow-up assessment	**Muscle weakness**	Referred 79% On NE 77%	Referred 64% On NE 82%	n.a.
	Myalgia	58%	56%	n.a.
	Myotonia	Referred 49% On NE 41% On EMG 81%	Referred 36% On NE 75% On EMG 90%	n.a.
Cataract		49%	60%	n.a.
Diabetes		30%	23%	n.a.
Heart		21%	22%	n.a.
Other systems		Dyslipidaemia 41% Thyroid dysfunction 32% Depression 21%	n.a.	n.a.
Laboratory assessments (n of patients)		230	150 (only CK and GGT)	n.a.
HyperCKaemia		176/230 (76%)	90%	n.a.
Hyper-GGT		112/205 (55%)	97/152 (64%)	n.a.
Low IgG		91/182 (50%)	13/20 (65%)	n.a.
Low Testosterone		23/39 (62%)	6/22 (29%)	n.a.
Low IgM		29/182 (16%)	2/20 (11%)	n.a.
Dyslipidemia		97/146 (66%)	n.a.	n.a.
High AST / ALT		AST 81/200 (41%) ALT 98/201 (49%)	n.a.	n.a.
EMG (n)		(216) Myotonic discharges 81% Normal 14%	(234) Myotonic discharges 90% Normal 10%	(117) no data
Muscle biopsy n (%)		77 (33%)	42	55 (41.5%)
Genetic testing (%)		100%	100%	71%

Table 3. Comparisons of DM2 clinical features among the largest cohorts of patients so far described.

The disease onset occurred at a mean age of 42±13.8 years without a significant difference between sexes (p=0.113). Most patients had a single symptom at onset (78.2%, n=231). Proximal weakness was the most frequent first symptom (55.4%) followed by myalgia (35.5%) and myotonia (25.4%). Symptoms at onset significantly differed between females and males (p<0.0001). Muscle weakness was more common in women (62.9%) than in men (43.8%) (p=0.001), while pain was more frequent in men (44.6%) than women (29.6%) (p=0.007). In addition, patients with weakness at onset were significantly older than those with myalgia, myotonia and pain associated with weakness (median 50, vs. 39, 31 and 35 years, respectively) (p<0.0001) (Fig. 3).

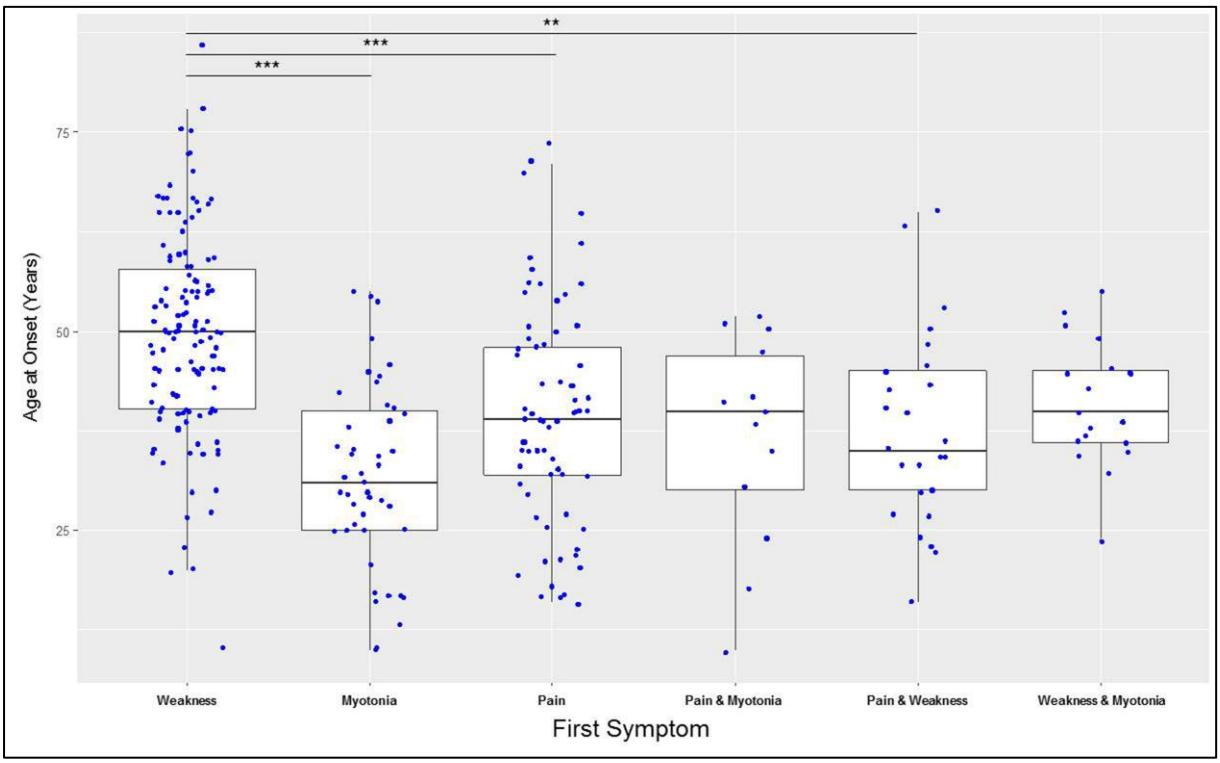

Fig. 3 Correlation between symptoms at onset and age at onset

A multinomial regression model revealed that age at onset and sex were significantly and independently associated with specific initial symptoms (p<0.0001 and p=0.002, respectively); being male was associated with higher odds of developing myalgia (OR 2.94 [95%CI 1.53-5.67], p=0.0012), while each additional disease year was associated with 10% lower odds of developing myotonia (OR 0.9 [95% CI 0.87-0.93], p<0.0001) and a 6% decrease in the odds of developing myalgia (OR 0.94 [95% CI 0.91-0.97], p<0.0001). At the time of the last follow-up assessment, patients often presented

several complaints simultaneously. Proximal weakness remained the most frequent symptom (79%), followed by myalgia (58%) and myotonia (49%).

Neurological examination (236 patients) showed that 77% of patients had a mild to moderate weakness in at least one muscle group. The most frequently affected muscles were neck (66%) and hip flexors (67%), followed by abdominal muscles (33%) and thumb extensors (31%) (Fig.4). Deep tendon reflexes (DTR) were normal in 60% of cases, brisk in 20% and reduced or absent in 17%. A calf hypertrophy was reported in 19 patients (8%). Myotonia was observed in 41% of patients.

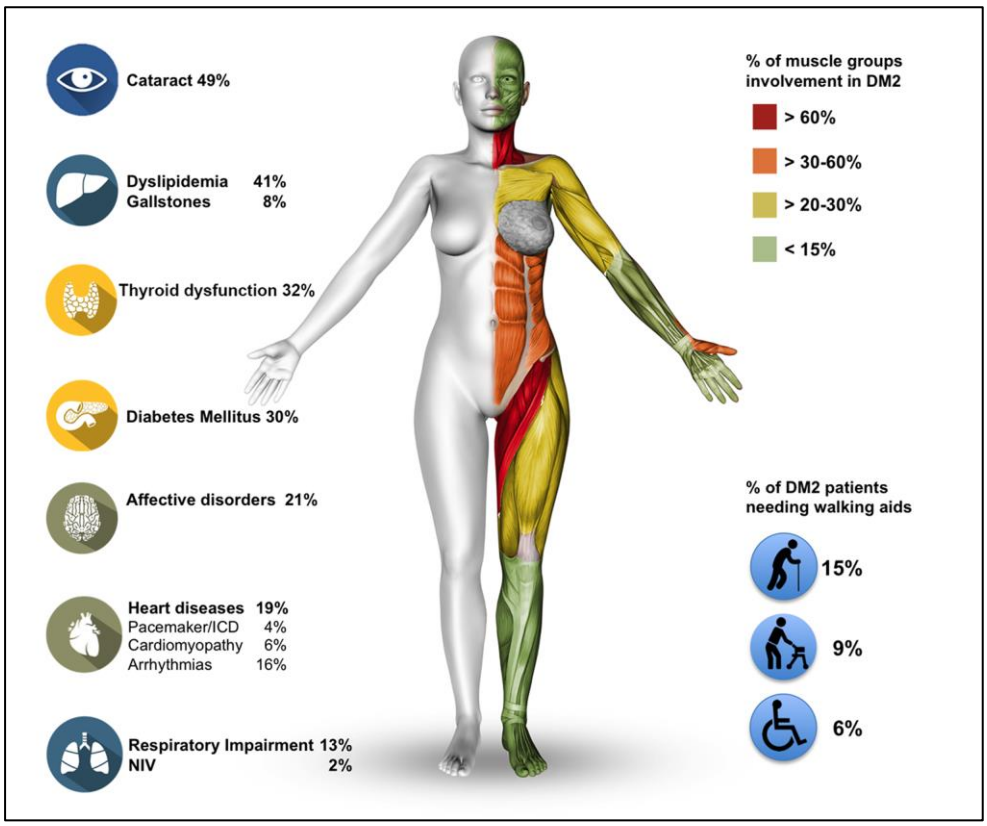

Fig. 4 Summary of the clinical features of this large DM2 cohort

Multisystemic involvement: The involvement of other systems and organs possibly related to DM2 is reported in Table 4 representing, in descending order, the most frequently detected comorbidities and the respectively age at onset, highlighting also differences between males and females (Fig. 5). Cataracts were reported in 49% of patients followed by hyperlipidemia (41%), hypertension (37%), thyroid dysfunction or surgery (32%), diabetes mellitus (30%), affective disorders (21%) (including depression and anxiety disorders), cardiac diseases (including cardiomyopathies and arrhythmias) (19%) and respiratory impairment (13%). Other organs and

systems (hearing loss, baldness, asthma bronchialis, tumours, skin, daytime sleepiness, gallbladder, stroke) were involved in less than 12% of cases.

Disease	Cohort n=307 (%)	Mean age (±SD)	Females n=186 (%)	Mean age (±SD)	Males n=121 (%)	Mean age (±SD)	χ^2
Cataract	151 (49%)	**50 (±12)**	104 (56%)	49 (±13)	47 (39%)	51 (±9)	**p=0,002**
Dyslipidemia	126 (41%)	52 (±10)	75 (40%)	54 (±9)	51 (42%)	51 (±11)	p=0,955
Hypertension	113 (37%)	55 (±9)	71 (38%)	57 (±8)	42 (35%)	53 (±10)	p=0,516
Thyroid dysfunction	99 (32%)	**44 (±13)**	72 (39%)	43 (±14)	27 (22%)	47 (±11)	**p=0,002**
Diabetes mellitus	92 (30%)	54 (±10)	51 (27%)	55 (±10)	41 (34%)	52 (±10)	p=0,239
Affective disorders	63 (21%)	**44 (±10)**	43 (23%)	45 (±10)	20 (17%)	41 (±9)	p=0,149
Heart diseases	60 (19%)	**50 (±14)**	35 (18%)	49 (±16)	25 (20%)	52 (±10)	p=0,792
Resp. Impairment	40 (13%)	51 (±15)	26 (14%)	49 (±17)	14 (12%)	55 (±10)	p=0,605
Hearing loss	36 (12%)	58 (±13)	25 (13%)	58 (±9)	11 (9%)	57 (±20)	p=0,194
Baldness	38 (12%)	**36 (±12)**	n.a.	n.a.	38 (31%)	36 (±12)	n.a.
Asthma bronchialis	37 (12%)	**27 (±12)**	24 (13%)	28 (±13)	13 (11%)	26 (±12)	p=0,559
Tumours	38 (12%)	53 (±14)	26 (14%)	52 (±13)	12 (10%)	54 (±16)	p=0,283
Skin changes	34 (11%)	**32 (±16)**	19 (10%)	33 (±15)	15 (12%)	31 (±17)	p=0,546
Daytime sleepiness	27 (8%)	54 (±9)	18 (10%)	51 (±8)	9 (7%)	60 (±8)	p=0,476
Gallstones	27 (8%)	47 (±14)	23 (12%)	47 (±13)	4 (3%)	47 (±20)	**p=0,006**
Stroke	15 (5%)	55 (±12)	7 (4%)	59 (±13)	8 (7%)	51 (±11)	p=0,255

Tab.4 Multisystemic Involvement in DM2: most frequent comorbidities and gender differences

Cataract, thyroid and gallbladder diseases occurred more frequently in women than in men (Fig. 5). No significant differences were found between males and females as regards the age at onset of these comorbidities.

Under “cardiac disease” we have considered a diagnosis of cardiomyopathy (n=10) or arrhythmias (n=50). Other complaints as coronary heart diseases (n=21 - 8%) or “occasional palpitations” referred in patients’ history but not supported by other clinical data (ECG, ultrasound, medications) were not considered. In total 13 patients had implanted an ICD or a pacemaker (4% of our cohort).

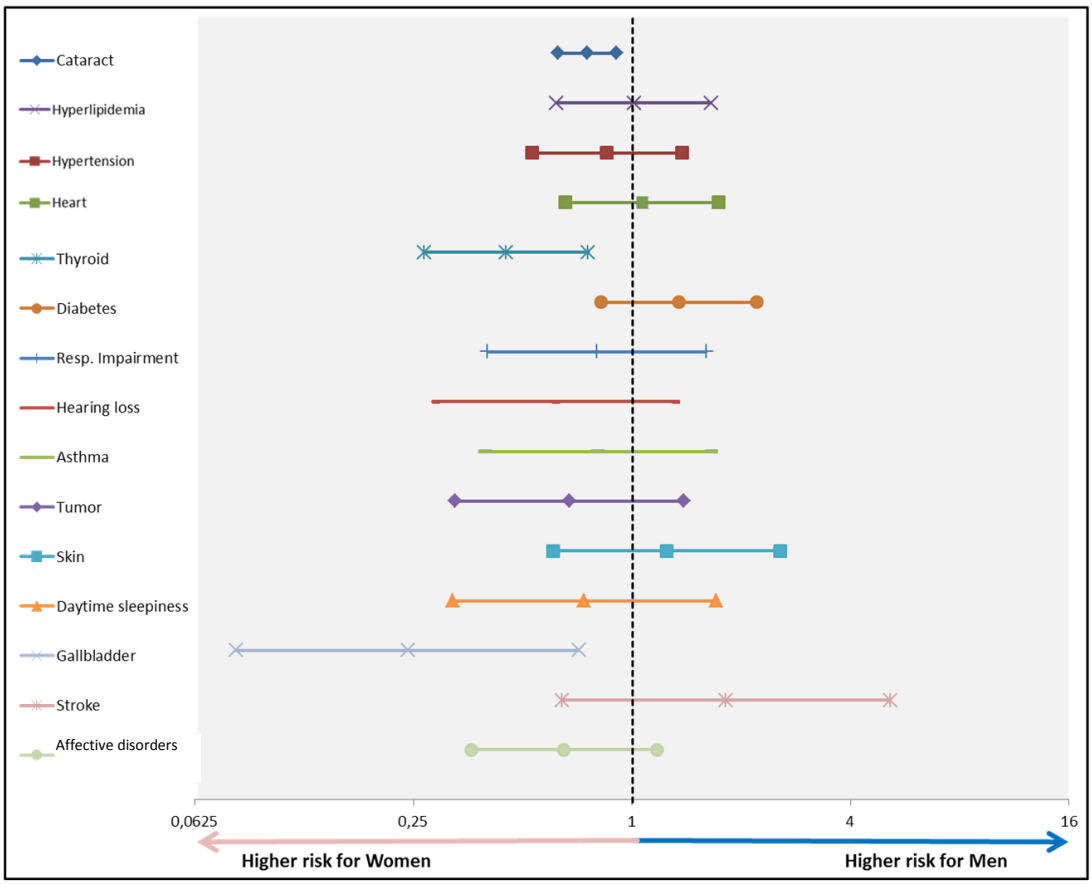

Fig.5 Gender specific differences of the multisystemic involvement in DM2*: Gender differences expressed as risk ratio (RR) of men/women with 95% confidence interval (CI). **Modified from Dogan et al 2016.*

The number of systems involved increased with aging (Spearmann p=0,0001; graphic: box plot) (Fig. 6) and females had a higher number of systems involved compared to males (p=0.027). Furthermore, an early onset of DM2 appears as an independent risk factor for the occurrence of systemic involvement (OR 0.945 [95%CI 0.905–0.987], p=0.011).

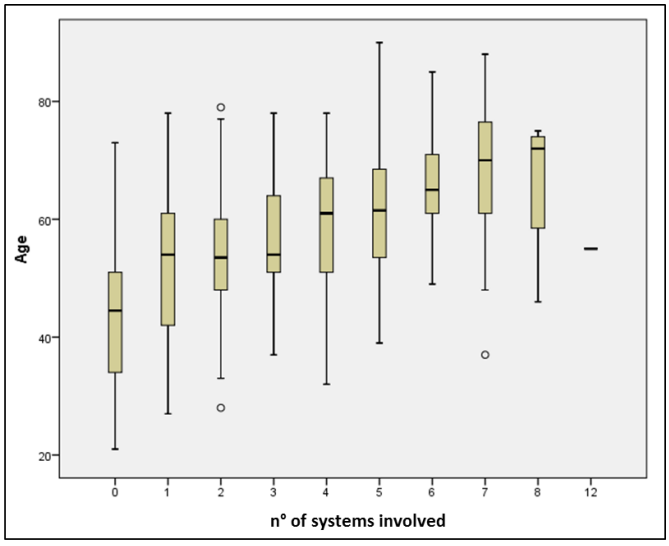

Fig.6 Correlation of multisystemic involvement to age in DM2: Correlation between number of systems involved and age of DM2 patients (Spearmann two-tails p=0,0001)

Considering the mean age at onset of each comorbidity, the diseases occurring at a younger age were: asthma, skin changes, baldness, affective disorders, thyroid dysfunction, heart diseases and cataract. Data confirmed also by analysing how many patients presented a given comorbidity before the age of 50 years (Tab.5).

Disease	Cohort n=307 (%)	Onset <50years
Cataract	151 (49%)	**68/151 (45%)**
Hyperlipidemia	126 (41%)	41/126 (33%)
Hypertension	113 (37%)	18/113 (16%)
Heart	101 (33%)	36/101 (36%)
Thyroid diseases	99 (32%)	**59/99 (60%)**
Diabetes mellitus	92 (30%)	27/92 (29%)
Affective disorders	63 (21%)	**36/63 (57%)**
Resp. Impairment	40 (13%)	14/40 (35%)
Hearing loss	36 (12%)	5/36 (14%)
Baldness	38 (12%)	**22/38 (58%)**
Asthma bronchialis	37 (12%)	**26/37 (70%)**
Tumours	38 (12%)	15/38 (39%)
Skin	34 (11%)	**20/34 (59%)**
Daytime sleepiness	27 (8%)	5/27 (18%)
Gallbladder	27 (8%)	11/27 (41%)
Stroke	15 (5%)	6/15 (40%)

Tab.5 Multi-systemic Involvement with onset before the age of 50 years.

At the time of **onset** of muscular complaints, a multisystemic involvement was found in 30% of patients (n=91/307) having at least one diagnosis among: diabetes (7%), cataract (14%), thyroid (10%) or cardiac dysfunction (8%). The remaining 70% (n=216) presented only muscular complaints.

4.4 Diagnostic Assessments:

For those patients who were examined at the FBI (n=236) we could evaluate how many of them performed, at the time of diagnosis, serologic assessments (97%), EMG (91%) and muscle biopsy (33%) (Fig. 7).

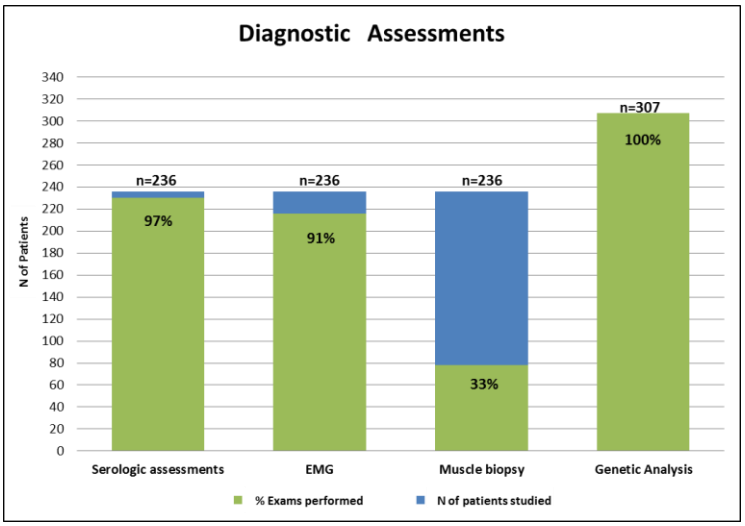

Fig.7 Diagnostic assessments performed at by DM2 patients at the time of diagnosis

4.4.1 Serologic Assessments:

Laboratory data were available for 230 DM2 patients (M = 100, F = 130) for a total of 505 laboratory exams. The mean age at laboratory assessment was 51 years old (±12), there was no statistically significant difference between males and females. The most frequently abnormal parameters were: CK (elevated in 76%), lipid profile (hyperlipidemia in 66%), testosterone (reduced in 62%), GGT (elevated in 55%), IgG (low in 50%), ALT (elevated in 49%), Vitamin D (reduced in 42%), AST (elevated in 41%), other abnormalities as low IgM, or IgA, elevated LDH, and hyper-parathormone were present in less than 20% of the cases (details in Tab.6 and Figure 8).

Parameter	N patients tested	N Abnormal (%)	Median (IQR;range)	Genders median (IQR)	p-Value Mann-Whitney U	% abnormal M/F	p-Value χ^2
CK (n.v. <170 UI/L)	230 (F=130, M=100)	176 (76%)	395 (397;171-5676)	M = 469 (425) F = 371(383)	**p=0,006**	M = 89 (89%) F = 87 (67%)	**p = 0,000**
Hyperlipidemia	146 (F=114, M=91)	97 (66%)	n.a.	n.a.	n.a.	n.a.	n.a.
Testosterone ng/dl	39(F=0, M=39)	23 (62%)	241 (±67.5)§	M =241 (±67.5)§ F = n.a.	n.a.	M = 23 (100%) F = 0	n.a.
GGT (n.v.<40 UI/L)	205 (F=114, M=91)	112 (55%)	69 (61;41-797)	M =70 (65) F= 69 (56)	**p=0,002**	M = 58 (64%) F = 54 (47%)	**p = 0,019**
IgG (n.v.800-1800mg/dl)	182 (F=104, M=78)	91 (50%)	681 (156; 308-797)	M =635 (141) F= 694 (132)	p = 0,686	M = 40 (51%) F = 51 (49%)	p = 0,475
ALT (n.v.<35 UI/L)	201(F=111, M=90)	98 (49%)	51 (22;36-312)	M =54(18) F= 44 (16)	**p = 0,0001**	M = 55 (61%) F = 43 (39%)	**P = 0,002**
Vit. D (n.v. 20-100 ng/ml)	84 (F=50, M=34)	35 (42%)	13 (4; 9-19)	M =13(4) F= 14 (3)	p = 0,443	M = 15 (58%) F = 20 (42%)	p = 0,593*
AST (n.v. <35 UI/L)	200(F=110, M=90)	81 (41%)	45 (12;36-216)	M =47 (13) F= 44 (10)	**p = 0,0001**	M = 46 (51%) F = 35 (32%)	**P = 0,006**
LDH (n.v.<250 UI/L)	133(F=77, M=56)	25 (19%)	285 (31;251-1242)	M =310 (51) F= 278 (28)	p=0,052	M = 12 (21%) F = 13 (17%)	p = 0,265*
PTH (n.v. 15-65 pg/ml)	129 (F=79, M=50)	23 (17%)	83 (18;67-148)	M =81(19) F= 83 (15)	p = 0,185	M = 10 (20%) F = 13 (16%)	p = 0,359*
IgM (n.v. 60-250 mg/dl)	182 (F=104, M=78)	30 (16%)	49 (9; 20-59)	M =47 (11) F= 50 (8)	**p = 0,029**	M = 18 (23%) F = 12 (12%)	p = 0,353*
IgA (n.v. 90-450 mg/dl)	182 (F=104, M=78)	12 low (6%) *6 high (3%)*	75 (8;19-85)	M =75 (6) F= 80 (5)	p = 0,325	M = 7 (9%) F = 5 (5%)	p = 0,589*

Tab.6 Abnormal serologic assessments. *= Fischer; §=normal distribution (mean±SD);

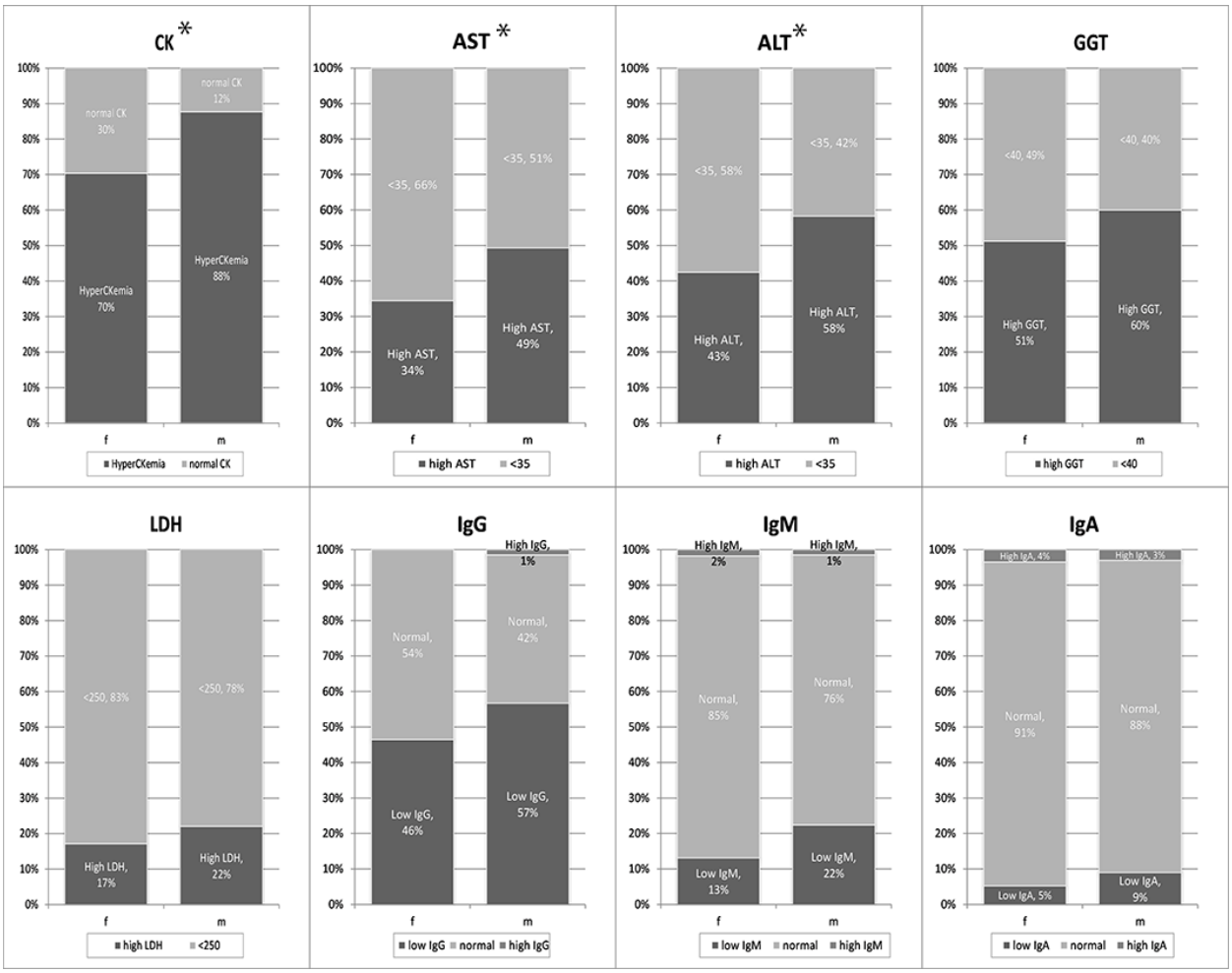

Figure 8 Relative distribution of serological parameters by sex (normalized to 100%). *=Significant differences were observed only for CK, ALT and AST more frequently abnormal in males than females (respectively p=0.0008, p=0.03 and p=0.037)

CK strongly correlated with AST, ALT and LDH but not with GGT (Fig. 9).

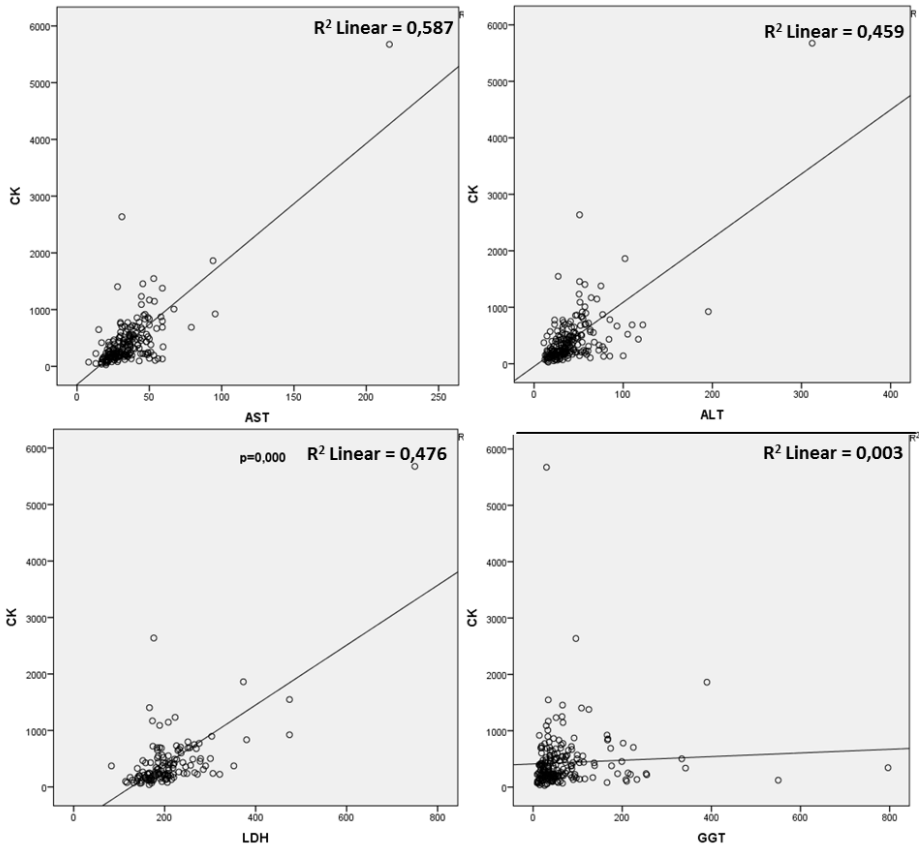

Fig. 9 Correlations between CK and AST, ALT, LDH and GGT (Pearson test)

4.4.2 Neurophysiological studies:

Neurophysiological data were available for 216/307 patients, all of them underwent at least the electromyography (EMG) and 114 of them also an electroneurography (ENG) at diagnosis. The EMG resulted normal in 31/216 patients (14%), myotonic discharges were detected in 176/216 patients (81%) either as isolated abnormality (n=91) or associated to myopathic (n=68) or neurogenic MUPs (n=17); a myopathic pattern without myotonic discharges or other pathological spontaneous activity was very rarely detected (7/216). An axonal sensorimotor polyneuropathy was detected in 27/114 patients (23%), the cause was considered a diabetes mellitus in 19/27 and unknown in the remaining 8 patients.

4.4.3 Muscle Biopsy

Data on muscle biopsy were available for 236 patients, those whose medical records were present in the FBI archives. A muscle biopsy was performed in 77/236 patients (33%). Histological features were typical of DM2 in 57/77 patients (74%), of the remaining 20 patients: 9 (12%) had a normal muscle biopsy, 3 (4%) mild neurogenic changes, 5 (6%) unspecific myopathic changes with some inflammatory infiltrates, 2 patients (3%) rimmed vacuoles and dystrophic changes, 1 patient a alterations suggestive of a myofibrillar myopathy (a panel diagnostic for myofibrillar myopathy causing genes was negative).

4.4.4 Genetic Analysis:

Only patients with a confirmed molecular diagnosis were included in the present study. In accordance with the recommendations for the genetic testing in DM2 (Kamsteeg *et al.,* 2012), in many cases the laboratories did not reported information on CCTG-repeat size that were available only for a minority of patients (<10%). For this reason, genotype-phenotype correlation could not be performed. Interestingly, three members of an Afghan family presented a homozygous CCTG-expansion without showing a severer phenotype in comparison to other heterozygous members of the same family. A detailed clinical description has been already published (Schoser *et al.,* 2004b). In some patients presenting a particularly evident myotonic reaction, the genetic analysis was broadened including also the search for mutation in those genes causing non-dystrophic myotonia (NDM), namely CLCN1 and SCN4A. Two patients showed known pathogenic mutations in the CLCN1 gene: the c.2680 C>T (p.894 R>X) in one patient (described in patients with autosomal dominant myotonia congenita) and the c.180+3A>T/c.501C>G in a compound heterozygous state (described in patients with autosomal recessive myotonia congenita). Both patients have been already described in the paper by Suominen et al in 2008 (Suominem *et al.,* 2008).

4.5 Diagnostic delay:

The mean age at diagnosis was 49 years (±12.6), with the oldest patient diagnosed at the age of 86 years old. No difference between males and females was observed (females 49±12.5; males 48.5±12.9; p=0,534). The time interval between onset of symptoms and diagnosis (diagnostic delay - DD) was, in our cohort, quite variable ranging from few months to 35 years (median 5, IQR 10 – mean 6.9±7.8). No significant difference was observed between men and women (p=0,058).

The diagnostic delay inversely correlated with age at onset (p=0,01) (Fig. 10) *(Spearman correlation test, one-sided:0,351 p-value=0,01)* but no statistically significant differences of diagnostic delay were observed considering the different muscular symptoms at onset (Kruskall Wallis H test: $\chi 2(2) = 0{,}940$, $p = 0.625$, with a mean rank diagnostic delay score of 103 for pain, 112 for weakness and 111 for myotonia). The presence of cataract or diabetes at onset was associated with shorter DD (p=0,018 and p=0,012 respectively – Mann Whytney U); similarly the presence of myotonic discharges on EMG and a typical muscle biopsy were associated with a shorter DD (p=0,031 and p=0,012).

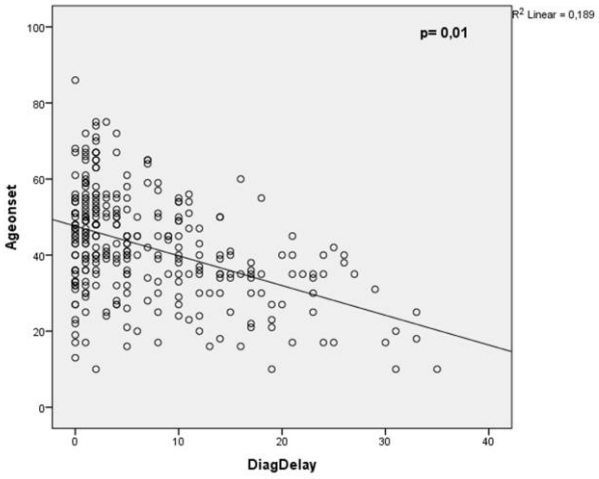

Fig.10 Correlation between the Diagnostic Delay (DD) and the age at onset

4.6 Overall disease course, progression, and prognosis

During the course of the disease 79 patients (26%) needed an assistive device (62% females and 38% males). More specifically, 48/307 patients (15%) adopted a walking cane at the mean age of 56 years (±8) (females 58%, males 42%), 28/307 (9%) used a rollator at the mean age of 62 years

(±11) (females 71%, males 29%) and 25/307 patients (6%) needed a wheelchair at the mean age of 56 years (±14) (females 72%, males 28%). 12/307 patients (4%) had a pacemaker or an ICD implanted at a mean age of 61 years (±8) (females 33%, males 67%).

5. DISCUSSION

I have herein described a large cohort of genetically confirmed DM2 patients, consisting of 249 families with 307 affected patients diagnosed between 2001 and 2016. The major determinants that allowed us to collect clinical data on such a large number of DM2 patients are the higher prevalence of DM2 in Germany and central Europe, the presence of a national patients´ registry for myotonic dystrophies and finally the key interest of the neurologists Kenneth Ricker and Benedikt Schoser. To date, two previous reports described the phenotype of a considerable number of DM2 patients (Hilbert et al., 2013; Day et al., 2003). Hilbert and colleagues analysed the clinical features and diagnostic delay of 135 DM2 patients, whose data were extracted from the U.S. Registry for myotonic dystrophies. In a study on DM2 patients of German and Northern American origin, Day et al. reported in 2003 clinical data of 234 patients. The primary aims of this study were the validation of an improved method to detect the DM2 expansion and the cohort description. A significant overlap between our patients and those included in the study by Day et al can be ruled out as only 21 patients of our study were diagnosed before 2003. Furthermore, the old clinical records of the German patients investigated in the Day study, the Kenneth Ricker DM2 archive, were still available for crosschecking. The reason why both largest DM2 cohorts so far described are mostly of German origin, might trace back to a founder mutation in middle Europe. Prevalence studies showed that DM2 is far more prevalent in Northern European countries where it seems as frequent as DM1 (Udd et al., 2006; Suominem et al., 2012). Furthermore, investigating on the origin of some of our DM2 families revealed, that a significantly higher proportion of their DM2-carrying ancestors, originated from Upper/Lower Silesia, a region that might show an unexpectedly higher prevalence of DM2. This unique observation certainly needs further verification with studies on DM2 prevalence in the non-Silesian Polish population. The reason why a disease originating from a common European founder haplotype (Liquori et al., 2003; Bachinski et al., 2003) shows such a discrepant prevalence across

Europe is still unclear. It has been hypothesized that historical and religious population bottlenecks and genetic drift may have caused such skewed frequencies in different sub-populations (Schoser et al., 2004b; Suominem et al., 2012).

What is the resulting updated clinical picture of DM2 patients? Comparing the clinical features of our cohort with both published large DM2 cohorts (Tab. 3), some interesting differences can be highlighted. The most striking divergence refers to the symptoms at onset; here we found that weakness was the most frequent complaint (55%), followed by myalgia (35%) and myotonia (25%). An inverted trend with notably higher occurrence of myotonia at onset (40%), and during the course of the disease, was reported by Day et al. This discrepancy might have two main reasons. Firstly, Day and colleagues selected their patients using the clinical criteria that preceded the advent of the genetic diagnosis, in which myotonia was considered among the inclusion criteria to suspect DM2 (Moxley et al., 2002). By selecting our patients on the basis of the confirmed genetic diagnosis, the clinical spectrum results more heterogeneous and less biased. Secondly, the mean age at symptoms onset was in our cohort higher (42 years vs. 37), this might also have reduced the occurrence of myotonia as we have found that age at onset is significantly associated with specific initial symptoms. In particular, patients with weakness were significantly older at onset than those with myotonia and pain (Fig. 3) and each incremental disease year was associated with a 10% drop for developing myotonia and a 6% decline for developing pain. This age-dependent worsening of weakness in a segmental progeroid disease as DM2 is not a novel finding, some recent studies have already highlighted analogies between the muscle degeneration occurring in DM2 and sarcopenia in a process mainly induced by satellite cells dysfunction (Malatesta et al., 2011, 2014; Renna et al., 2014; Mateos-Aierdi et al., 2015). We have also found that proximal muscle weakness was more frequent and severe in women; in fact, a higher proportion of women also needed walking aids during the disease course. The age-related reduced concentration of sex hormones, in particular after the menopause, is among the key mechanism for sarcopenia and muscle weakness in women who, according to the MYOAGE-project, are more subjected than men to decrements in muscle function and quality (Sipilä et al., 2013).

The reduced prevalence of myotonia with ageing is a new finding and opens a diagnostic avenue for older paucisymptomatic DM2 patients within the spectrum of unclassified limb girdle

weakness. This is an important issue, as especially with recent exome and genome sequencing techniques repeat disorders remain undetected. A common consideration is the effect of total muscle mass and myotonia. By increase of sarcopenia and muscle degeneration beyond the age of 40 years, clinical myotonia declines in parallel, as seen in congenital myotonia. It might be hypothesized that, with time, muscle degeneration and weakness might mask or replace myotonia caused by CLC-1 and, rarely, SCNA4 channels dysfunction (Ursu et al., 2012; Bugiardini et al., 2015). Furthermore, the classical percussion myotonia at the thenar eminence is not a frequent finding in our clinical practice, whereas it might be observed in more proximal muscles such as forearm muscles, a phenomenon already reported in the literature (Johnson and Heatwole, 2013).

The presence of myalgia in DM2 patients is a recurrent finding; our study showed a lifetime prevalence of pain of about 59%. Its presence did not correlate with myotonia or weakness, and was a frequent presentation of the disease in young males. Little is known about its pathophysiology; some authors hypothesized an association with myotonia of deep muscles, this explanation is however not convincing, as DM1 patients presenting more myotonia have indeed less pain (Suokas et al., 2012). Alternatively, the musculoskeletal imbalance secondary to muscle weakness has also been advocated as possible causing factor for pain, however this would not explain the occurrence of pain as early symptom in DM2 in the absence of muscle weakness (George et al., 2004). Novel insights on the pathophysiology of pain has been provided by Moshourab et al; in this elegant study they performed quantitative sensory testing in myalgia and non-myalgia DM2 patients and compared these clinical results with the transcriptome profiles of muscle biopsy specimens of the patients. They found that distinct transcriptome profiles differentiated myalgia from non-myalgia patients thus suggesting that myalgia might be initiated and maintained by specific molecular changes within the muscle. In myalgic DM2 patients, the highest differential expression was a decrease in the levels of monoamine oxidase A (MAOA) and a significantly increased expression of CYB5D1, GSTCD, GRB14, PANK1, ZNF711, FAM26E, PFKFB2, ZNF841, HECW2, SLC16A12, FRMPD1, NR4A3 and SLC16A12. (Moshourab et al., 2016)

Our study assessed for the first time simultaneously the involvement of multiple systems in many DM2 patients also evaluating gender differences (Montagnese et al., 2017). The muscular

system is the most frequent and earliest affected system, about 70% of patients present at onset purely muscular complaints. Other frequent comorbidities were, in order of frequency: cataract (49%), dyslipidaemia (41%), hypertension (37%), thyroid dysfunctions (32%), diabetes (30%), affective disorders (21%), and heart diseases (19%).

The occurrence of cataract, diabetes and cardiac diseases was similar to what reported in former DM2 cohorts (Day et al., 2003; Ricker et al., 1995; Meola and Cardani, 2015) (Tab. 3). The increased risk for cataract in our female patients suggests a possible pathogenetic role of sex hormones. In the general population, many epidemiological studies on cataract are performed on elderly (>60 yrs.) (Prokofyeva et al., 2013) and the higher prevalence of cataract in women has mostly been found in the postmenopausal period (Kanthan et al., 2010), as oestrogen exert a protective effect on "cataractogenesis" (Zetterberg and Celojevic, 2015). In our study, however, also considering only the group of patients younger than 50 years, pre-menopausal age, females still presented a significantly higher prevalence of cataract (p=0,04). Similar results have been found in DM1 (Dogan et al., 2016).

A meta-analysis estimated that the mean prevalence of thyroid dysfunctions in the general European population was 3.82% (95% CI, 3.77%–3.86%) and a female preponderance was observed in all the analysed studies (Garmendia Madariaga et al., 2014). According to our results, the prevalence of thyroid dysfunction in DM2 patients (mostly hypothyroidism) seems even higher as observed in DM1 (32% vs. 21%) (Dahlqvist et al., 2015) and a careful monitoring is advisable to prevent hormonal imbalances that may worsen clinical manifestations (Sansone et al., 2000).

Albeit the role of DM2 in determining an increased female-prevalence for cataract and thyroid diseases has yet to be elucidated, adopting gender-specific prevention and screening protocols for DM2 comorbidities should be considered.

A dyslipidaemia was reported in our patients twice as frequently as the general German population (GEDA 2010-2012;http://www.geda-studie.de/deutsch/ergebnisse/ergebnisse-nach-themen/chronische-erkrankungen.html) (41% vs. 20.2%). To the best of our knowledge, studies assessing the lipid metabolism in DM2 have not been performed, however in some DM1 cohorts a higher occurrence of dyslipidaemia, both hypercholesterolemia and hypertriglyceridemia, was found

(Fernandez-Real et al., 1999; Heatwole et al., 2006; Vujnic et al., 2015). Hypertension prevalence in our patients (37%) was similar to the general population (30-33%) (Neuhauser et al., 2013). Conversely, affective disorders appeared to occur more frequently in DM2 than in the general population (21% vs. 8%). This might be ascribed to the significant correlation with the presence of pain ($p=0.0014$, $\chi 2$-test). A high prevalence of depression in DM2 patients (50%) has indeed also been found in a study combining neuropsychological tests with fMRI and voxel-based morphometry, where a correlation with brain structural abnormalities was demonstrated (Minnerop et al., 2011, Schneider-Gold et al., 2015). The percentage of patients with respiratory impairment (13%) was similar to what reported by different researchers (between 6-15%) (Sansone and Gagnon, 2015). Sleep disturbances were reported for 28 of our patients (9%), of them 15 were diagnosed with restless legs syndrome (RLS). These data probably underestimate the prevalence of sleep disorders in DM2. In fact, some previous studies found that a significantly higher proportion of DM2 patients have poor sleep quality in comparison to healthy controls (Lam et al., 2013; Romigi et al., 2014); RLS was reported in up to 60% of the patients (Lam et al., 2013) and obstructive sleep apnoea (OSAS) in up to 58% of patients (Romigi et al., 2014). Further studies systematically assessing sleep quality in larger cohorts are needed to better define the prevalence and the characteristics of sleep disorders in DM2.

Due to the described heterogeneous spectrum of clinical symptoms, the road to the diagnosis can be very long for many patients, and some authors have described this path as a "diagnostic odyssey" (Hilbert et al. 2013). We have retrospectively evaluated extensive serologic assessments performed in a large subgroup of our patients. Our results, in addition to those of previous studies (Heatwole et al., 2011, Day et al., 2003), could confirm and reinforce the pattern of laboratory abnormalities observed in DM2 patients, namely hyperCKaemia (76%), dyslipidaemia (66%), elevated GGT (55%), low IgG (50%), elevated ALT (49%) and AST (41%). Except for a higher occurrence of elevated CK, AST and ALT in males compared to females no other significant gender differences were observed. Similarly, no differences between females and males were observed about the results on the neurophysiological studies or muscle biopsy.

The mean age at diagnosis in our cohort was 49±12.6 years with a diagnostic delay of 6.9 years. This is about the half compared to the American data (14 years) (Tab. 3) (Hilbert et al., 2013).

A likely explanation is, as discussed, the higher prevalence of DM2 in Germany together with the increased disease awareness and the direct access to genetic testing. This latter aspect emerges also from the lower percentage of muscle biopsies performed in our patients (33% vs. 41.5%) (Hilbert et al., 2013), thus suggesting that clinical, laboratory, and neurophysiological data alone were sufficient in about 70% of patients in having the diagnostic suspect and performing the appropriate genetic testing.

Among the strengths of this study, besides the high number of patients, is their relative homogeneous geographical origin that facilitates the comparison with the general population; furthermore, the absence of significant age differences between males and females allowed gender's comparisons.

A major limitation of our study is the retrospective design of the analysed data together with the cross-sectional nature of data collected by postal surveys. However, I have restricted our cohort of originally more than 400 genetically proven DM2 patients to 307 patients, where the most reliable data-set was available. Furthermore, the collection of information has been probably affected by the accuracy of different doctors in record keeping, so that the prevalence of some comorbidities might have been underestimated.

6. CONCLUSIONS

In conclusion, I have provided an updated clinical description of DM2 after 15 years of the causative gene description in 2001 and of the description by Day and colleagues in 2003. Myotonia seems to occur less frequently in comparison to previous studies, whereas proximal and axial weakness is the leading core symptom at onset and during the disease. With ageing, there is a tendency towards the worsening of the muscle weakness, whereas other complaints as pain and myotonia tend to decrease. Gender differences could be identified: females show more frequently muscle weakness, multisystem involvement, and need of using walking aids. For these reasons, the clinical picture of DM2 seems to be more severe in women than men. Easier access combined with a lower threshold for genetic testing will help to reduce the huge diagnostic delay in DM2. These new clinical aspects

should be considered in the present care and monitoring, and for the planning of future clinical trials in DM2.

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SUPPLEMENTARY MATERIAL

QUESTIONNAIRES:

Questionnaire n°1 "Survey on the origin of DM2 patients´ families" *(Att. 1):* 256 DM2 patients received this questionnaire where they were asked to provide the place of birth of their parents and grandparents and to indicate which family branch transmitted the disease (if known).

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Patient/Patientin (Name, Vorname, Geburtsdatum):

Geburtsort (Stadt, Region, Land):

Wer hat/hatte möglichst in Ihrer Familie die Krankheit? Mutter ☐ Vater ☐

Eltern des Patienten/der Patientin
Geburtsort der Mutter (Stadt, Region, Land):
Geburtsort des Vaters (Stadt, Region, Land):

Großeltern des Patienten/der Patientin (mütterlicherseits)
Geburtsort der Großmutter (Stadt, Region, Land):
Geburtsort des Großvaters (Stadt, Region, Land):

Großeltern des Patienten/der Patientin (väterlicherseits)
Geburtsort der Großmutter (Stadt, Region, Land):
Geburtsort des Großvaters (Stadt, Region, Land):

Questionnaire n°2 "General clinical information on DM2 patients" *(Att. 2):* it is a DM-oriented questionnaire developed by Moxley R., that has already been used in other previously performed studies at the FBI. 312 patients received this questionnaire per post.

Allgemeiner Fragebogen

(Falls sie mehr Platz benötigen als in den Spalten vorhanden ist, dürfen sie gerne die Rückseite benützen!)

Ich leide unter: □ Myotoner Dystrophie 1
□ Myotoner Dystrophie 2 / PROMM

Allgemeine Daten zu Ihrer Person:

Datum:_____

Name(Nachname,Vorname):_____

Adresse:_____

Telefonnummer:_____

E-Mail:_____

Geburtsdatum:_____

Geschlecht: □ weiblich □ männlich

Name des Hausarztes:_____

Adresse des Hausarztes:_____
_____

Telefonnummer des Hausarztes, bzw des betreuenden Arztes: _____

Ihre aktuelle **Größe**:_____cm, Ihr aktuelles **Gewicht**:____kg

Information über Ihre Diagnosestellung:

1. Wegen welchen Beschwerden haben sie sich bei einem Arzt vorgestellt, als er Ihre Myotone Dystrophie/ PROMM diagnostiziert hat? (Was waren Ihre ersten Krankheitsanzeichen?)

□ keine Beschwerden, aber bei einem Verwandten hatte man die Krankheit kürzlich entdeckt

□ körperliche Symptome(zum Beispiel Muskelschwäche, Herzbeschwerden, Sehstörungen oder andere Auffälligkeiten)

□ In den Blutwerten haben sich Veränderungen gezeigt. Wenn ja, dann bitte welche?

- Wie alt waren sie, als sie zum ersten Mal Beschwerden (erste Krankheitsanzeichen) hatten? _____ Jahre

- Wie alt waren sie, als die Erkrankung bei Ihnen festgestellt wurde? _____ Jahre

2. Hatten sie Untersuchungen wie die aufgezählten *(bitte zutreffendes ankreuzen):*

□ Untersuchung eines Neurologen(Nervenfacharzt)?

□ Elektromyographie (EMG, Nadeln in der Muskulatur, um die elektrischen Aktivitäten zu messen)?

□ Muskelbiopsie (Entnehmen eines Stückens des Muskels zur feingeweblichen Untersuchung)?

□ DNA- Test (Bluttest auf Gene, die verändert sind)?

3. Welche Untersuchung hat bei Ihnen die Krankheit bewiesen?

---

4. Wer hat bei ihnen die Myotone Dystrophie festgestellt?
□ Hausarzt □ ein Neurologe □ sie selbst
□ ein Familienmitglied
□ ein Spezialist für vererbte Muskelkrankheiten

5. Sind sie der erst bei Ihrer Familie, bei dem die Krankheit aufgetreten ist?
□ **Ja** □ **Nein**

6. Ist jemand anderes Ihrer Verwandtschaft, bzw. Ihrer Familie betroffen?

□ **Ja** □ **Nein** □ **Nicht sicher**

Falls Ja, bitte angeben

	ja	nein	Nicht sicher	Anzahl der Erkrankten
Bruder und Schwester				
Kinder unter 18 Jahre				
Kinder über 18 Jahre				
Mutter				
Vater				
Großeltern				
Onkel und Tante				
Cousinen, Cousins, andere Verwandte				

Angaben zu Sozialem Umfeld:

1. Womit sind sie momentan beschäftigt?
□ Berufstätig als:_____
□ Hausfrau, Hausmann
□ Student
□ Rentner
□ Arbeitsunfähig aufgrund der Myotonen Dystrophie
□ Arbeitsunfähig aufgrund anderer Ursache
□ Arbeitslos
Kommentare: _____

2. Hat sie die Myotone Dystrophie in Ihrer Tätigkeit eingeschränkt?

□ **Ja** □ **Nein**

Falls ja:

	Ja	**Nein**
Wurde die Arbeit auf die neuen Bedürfnisse abgestimmt?		
Haben Sie ihren Job verloren?		
Sind Sie in Frühente gegangen?		
Anderes*(bitte schildern)*_____ _____		

3. Bitte geben sie an, welchen Abschluss und Schulabschluss sie erworben haben:

- □ Akademischer Titel
- □ Ausbildung
- □ Hauptschulabschluss
- □ Realschulabschluss
- □ Abitur

□ keinen
□ eine anderen: _____

4. Benützen sie technische Hilfsmittel aufgrund der Myotonen Dystrophie?

	Ja	**Nein**	Alter, als das Hilfsmittel erstmals benötigt wurde
Fußgelenksstützung			
Lange Stützschienen für die Beine			
gelegentlich Gehstock			
Gelegentlich Gehwagen/ Rollator			
Rollstuhl 1. nur für lang Wege			
2. oft, auch für kurze Strecken			
3. Immer			
Atemhilfe (CPAP oder BIPAP)			
Beatmungsgerät			
Herzschrittmacher			
Andere: _____			

Anzeichen und Symptome:

	Ja	**Nein**	Alter, als die Probleme angefangen haben
Probleme mit den Händen, dem Greifen, oder Steifigkeit der Hände			
Schwierigkeiten eine feste Faust zu machen, diese zu öffnen, oder Probleme Dosen oder Gläser zu öffnen			
Probleme deutlich zu sprechen			
Probleme beim Schlucken			
Schwäche der Gesichtsmuskulatur			
Probleme auf den Zehenspitzen zu gehen, auf den Fersen zu gehen, oder Instabilität im Fußknöchel mit leichtem Umknicken			
Schwierigkeiten aus dem Sitzen aufzustehen, vom Boden aufzustehen			
Probleme beim Treppensteigen			
Probleme beim Atmen, oder Atemnot			
Katarakt, grauer Star, Augenlinsentrübung			
Haarausfall			
Schneller Herzschlag, unregelmäßiger Herzschlag, Herzklopfen oder Schrittmacher			

Medikamente

Nehmen Sie Medikamente ein? □ **Ja** □ **Nein**
Wenn Ja, geben Sie bitte den Namen aller rezeptpflichtigen und rezeptfreien Medikamente, sowie aller natürlichen Präparate und Nahrungsergänzungsmittel die sie einnehmen an.

Name des Medikaments	seit wann nehmen Sie das Medikament ein	Milligramm pro Tablette	Tabletten pro Tag

Allergien

Haben Sie eine Allergie gegen bestimmte Lebensmittel oder Medikamente?
Wenn ja, gegen was:

Rauchen Sie? □ **Ja** □ **Nein**

Wenn Ja, wie viele Zigaretten pro Tag: _____ seit wann: _____

Trinken sie Alkohol? □ **Ja** □ **Nein**

Wenn Ja, wie viel? _____

Behandlungen

Haben Sie schon einmal eine der folgenden Behandlungen bekommen?

	Ja	**Nein**	**Weiß ich nicht**
Physikalische Therapie			
Genetische Beratung			
Psychologische Beratung			
Sprach Therapie			
Ergotherapie/ Beschäftigungstherapie			
Atemtherapie			
Andere			

Andere Erkrankungen

Haben oder hatten sie schon einmal eine der folgenden Erkrankungen oder Symptome?

□ Diabetes
□ erhöhter Blutdruck
□ Asthma
□ Atemmuskelschwäche
□ Schilddrüsenbeschwerden
□ Rheumatische Arthritis
□ Lungenemphysem (Lungenüberblähung)
□ Gallenblasenbeschwerden
□ Pneumonie (Lungenentzündung)
□ Herzerkrankung Herzschlagunregelmäßigkeiten
□ Krebs oder Tumor, welcher_____
□ chronisch Infektion
□ Leberbeschwerden
□ Erhöhte Cholesterinwerte
□ Refluxerkrankung
□ ein Kind das Symptome der Myotonen Dystrophie innerhalb der ersten vier Lebenswochen gezeigt hat
□ psychologische Probleme wie Depressionen und Angststörungen
□ andere: _____
_____

□ Schlaganfall
□ Nierenbeschwerden
□ Ateminsuffizienz
□ Fehlgeburt
□ Totgeburt
□ Magengeschwür
□ Verstopfung
□ Impotenz
□ Prostatabeschwerden

Knochenbrüche

Bitte listen sie alle Knochenbrüche auf, die sie jemals hatten

Bruch	Wann ist das gewesen	War die Myotone Dystrophie zu diesem Zeitpunkt schon bekannt	
		ja	nein

Fettstoffwechselfragebogen
(Bitte alle Fragen beantworten, auch wenn es Ihnen so vorkommt als würden wir sie alles mehrfach fragen!
Falls sie nicht genügend Platz in den Spalten haben, benützen Sie bitte die Rückseite!)

	Ja	Nein	Wenn ja, dann bitte angeben seit wann
Ist bei ihnen eine Arteriosklerose (Arterienverkalkung) bekannt?			
Ist bei ihnen eine Verkalkung der Herzkranzgefässe oder Halsschlagader bekannt?			
Ist bei ihnen schon einmal ein Herzinfarkt aufgetreten?			
Ist bei ihnen schon einmal ein Schlaganfall aufgetreten?			
Haben sie Schwierigkeiten weite Strecken zu gehen, weil ihre Beine schlecht durchblutet sind? (sie also Schmerzen in den Beinen bekommen)			
Wenn ja, wie viele Meter können sie gehen, ohne stehen zu bleiben?_____			
Hatten sie schon einmal eine Bauchspeicheldrüsenentzündung?			
Leiden sie unter einer Leberverfettung?			
Hatten sie schon einmal, bzw. haben sie gelbe, knubbelige Fetteinlagerungen in der Unterhaut, wie z.B. an den Augen, an Sehnen der Hände oder Füße, zwischen den Fingern?			

- Leiden sie unter Zuckerkrankheit(Diabetes mellitus)? □ **Ja** □ **Nein**

Falls Ja: □ Typ 1 oder □ Typ 2(Alterszucker)
□ Insulinspritzen nötig
□ nur Medikamente nötig

Wie war Ihr letzter HBA1c –Wert(Langzeitzucker): _____
(Fragen sie gegebenenfalls ihren Hausarzt!)

- Leiden sie unter Bluthochdruck? □ **Ja** □ **Nein**

Falls Ja: Wie hoch sind die Werte, die sie normalerweise messen? _____

Haben sie Medikamente für das Problem?
□ **Ja** □ **Nein**

Name des Medikaments	Dosierung in **mg**	Seit wann nehmen sie es	Wenn sie es nicht mehr nehmen, über welchen Zeitraum sie es genommen haben

Wie sind die Werte unter Therapie? □ im Sollbereich
□ zu hoch
□ zu niedrig

- Sind bei ihnen in Blutuntersuchungen schon einmal erhöhte Fettwerte oder erhöhtes Cholesterin aufgefallen? (z.B. Cholesterin, Triglyceride, LDL, VLDL, HDL, IDL, Chylomikronen)

□ **Ja** □ **Nein**

Falls Ja: - Wann zum ersten Mal? _____

- Welche Werte waren erhöht? _____

- Wie hoch genau waren diese? (gegebenenfalls beim Arzt erfragen) _____

- Haben sie schon einmal, oder als Reaktion auf eine Blutfetterhöhung fettsenkende Medikamente von Ihrem Arzt verordnet bekommen?

□ **Ja** □ **Nein**

- Haben sie diese Medikamente wieder abgesetzt?

□ **Ja** □ **Nein**

Wenn ja, warum, und was für Veränderungen waren genau der Grund für das Weglassen der Tabletten?
_____
_____
_____

Falls sie eines der Medikamente <u>einnehmen oder schon einmal eingenommen haben</u>, dann bitte ankreuzen! Falls sie sich nicht sicher sind, ob Ihr Medikament zu den unten genannten Wirkstoffgruppen gehört, bitte in der Packungsbeilage nachsehen <u>welche Wirksubstanz</u> enthalten ist, und diese in die leere Spalte eintragen! Vielen Dank!
(sonst einfach Name in leere Spalte eintragen und ebenfalls Dosierung angeben)

	Ja	Dosierung in mg	Seit wann nehmen sie das Medikament	Nicht mehr: für wie lange haben sie die Medikamente eingenommen?	Warum nehmen sie es nicht mehr ein?
Lovastatin bzw.Mevinacor					
Pravastatin					
Simvastatin z.B. Zocor					
Atorvastatin z.B. Sortis					
Andere HMG-CoA Reduktase-hemmer (CSE-Hemer) z.B. Locol					
Fenofibrat z.B.Lipidil					
Gemfibrozil z.B.Gefilon					
Bezafibrat z.B. Cedur					
Andere Fibrate z.B. Lipomerz					

- Zu dem Zeitpunkt, als sie die Medikamente eingenommen haben, hat einer der folgenden Punkte auf sie zugetroffen? (Bitte die Antwort aufschreiben)

1. Wie alt waren sie? _____

2. Litten sie an Schilddrüsenunterfunktion? _____

3. Litten sie an einer Lebererkrankung, oder hatten sie erhöhe Leberwerte?
_____

4. Haben ihre Nieren nicht gut gearbeitet, oder waren die Nierenwerte erhöht?
_____

5. Hatten sie einen Infekt (Durch Bakterien oder Viren hervorgerufene Krankheit)?
_____

6. Hatten sie gerade eine Operation hinter sich? _____

7. Hatten sie gerade eine größere Verletzung oder einen Unfall gehabt?
_____

8. Haben sie mehrere Fettsenker gleichzeitig verordnet bekommen? Wenn ja,
welche!_____
_____

- Sind ihre Beschwerden der Myotonen Dystrophie durch diese Tabletten in irgendeiner Weise beeinflusst worden?

□ **Ja** □ **Nein**

Falls ja:

Beschwerden	Was hat sich verändert

- Wurden Laborwerte (Muskelspezifische Werte des Blutes, wie z.B. CK) bestimmt, seit dem sie diese Tabletten nehmen, oder in der Zeit, als sie die Fettsenker eingenommen haben?

□ **Ja** □ **Nein**

- Wie haben sich diese verändert? _____

- War der CK-Wert bei Einnahme der Fettsenker in ihrem Blut erhöht? (Wenn Sie das nicht wissen, fragen sie bitte Ihren Arzt, lassen sie sich die Ergebnisse geben und legen sie diese bei!)

□ **Ja** □ **Nein**

Falls ja, wie hoch war er, und wie hoch war er vor der Therapie mit Fettsenkern	
Vorher	Unter Therapie

- Haben sich das Cholesterin durch die Tabletten gesenkt?

□ **Ja** □ **Nein**

Falls ja, von _____ auf_____ (Bitte gegebenenfalls beim Arzt erfragen! Danke!)

- Haben sie Nebenwirkungen der Tabletten bekommen?

□ **Ja** □ **Nein**

Falls ja, welche waren das:

- Hatten sie vermehrt Muskelschmerzen?

□ **Ja** □ **Nein**

Falls ja, bitte ankreuzen was zutrifft:	
Vorherige Muskelbeschwerden sind schlimmer geworden	
Neue Muskelschmerzen sind dazugekommen	
Wurden daraufhin Blut und Urinuntersuchung durchgeführt? (Falls ja, bitte angeben von welchem Arzt, damit wir gegebenenfalls die Werte einsehen können) _____	

- Ist eine Rhabdomyolyse (Muskelzersetzung) bei ihnen dadurch hervorgerufen worden? □ **Ja** □ **Nein**

- Haben sie daraufhin die Therapie mit Fettsenkern beendet? □ **Ja** □ **Nein**

- Hat sich die Myotone Dystrophie bei ihnen durch die Einnahme dieser Tabletten verschlechtert? □ **Ja** □ **Nein**

- Hat sich sonst in der Zeit, in der sie diese Fettsenker genommen haben an ihrer Medikation, etwas verändert?□ **Ja** □ **Nein**

- Hat sich an Ihren Lebensumständen (vermehrt Stress, Scheidung, Jobverlust, …) etwas geändert, während sie die Fettsenker eingenommen haben? □ **Ja** □ **Nein**

- Hatten sie einen viralen oder bakteriellen Infekt gehabt, zu der Zeit, als sie die Nebenwirkungen gemerkt haben?□ **Ja** □ **N**

- Haben sie sich in dieser Zeit stärker Körperlich belastet als normalerweise(vermehrt Sport, viel Bewegung)? □ **Ja** □ **Nein**

- Ist bei ihnen eine rheumatische Erkrankung bekannt? □ **Ja** □ **Nein**

Falls ja, welche: _____

- Hatten sie in der Zeit, in der sie die Fettsenker eingenommen haben, besonders Schmerzen und Steifigkeitsgefühl im Schultergürtelbereich? □ **Ja** □ **Nein**

- Haben sie Kortison von ihrem Arzt bekommen, die die Beschwerden gelindert haben? □ **Ja** □ **Nein**

- Hatten sie gleichzeitig eine schmerzhafte Schläfe und Sehstörungen? □ **Ja** □ **Nein**

- Leiden sie unter Fibromyalgie? □ **Ja** □ **Nein**

- Falls sie mit Beschwerden auf die Fettsenkertabletten reagiert haben, wurde von Ihren Muskeln ein Stückchen (Biopsie) genommen, und feingeweblich untersucht? □ **Ja** □ **Nein**

Falls ja, aus welchem Körperteil, und was war der Befund? (Den Befund bitte vom Arzt geben lassen, und beilegen! Damit ersparen sie uns viel Mühe! Vielen Dank!)

Welcher Arzt hat die Probe entnommen?

_____

Eidesstattliche Versicherung

Montagnese, Federica

Name, Vorname

Ich erkläre hiermit an Eides statt,

dass ich die vorliegende Dissertation mit dem Thema

Die klinischen und diagnostischen Merkmale der Myotonen Dystrophie Typ 2: retrospektive Studie eines großen Patientenkollektivs

selbständig verfasst, mich außer der angegebenen keiner weiteren Hilfsmittel bedient und alle Erkenntnisse, die aus dem Schrifttum ganz oder annähernd übernommen sind, als solche kenntlich gemacht und nach ihrer Herkunft unter Bezeichnung der Fundstelle einzeln nachgewiesen habe.

Ich erkläre des Weiteren, dass die hier vorgelegte Dissertation nicht in gleicher oder in ähnlicher Form bei einer anderen Stelle zur Erlangung eines akademischen Grades eingereicht wurde.

München, 20.11.2018

Ort, Datum

Federica Montagnese

Unterschrift Doktorandin/Doktorand