CHAPTER 18

MYOTONIC DYSTROPHY TYPE 1 OR STEINERT'S DISEASE

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Abstract: Myotonic Dystrophy Type 1 (DM1) is the most common worldwide autosomal dominant muscular dystrophy due to polynucleotide $[CTG]_n$ triplet expansion located on the 3′UTR of chromosome 19q13.3. A toxic gain-of-function of abnormally stored RNA in the nuclei of affected cells is assumed to be responsible for several clinical features of the disease. It plays a basic role in deregulating RNA binding protein levels and in several mRNA splicing processes of several genes, thus leading to the multisystemic features typical of DM1. In DM1, the musculoskeletal apparatus, heart, brain, eye, endocrine, respiratory and gastroenteric systems are involved with variable levels of severity. DM1 onset can be congenital, juvenile, adult or late. DM1 can be diagnosed on the grounds of clinical presentation (distal muscular atrophy and weakness, grip and percussion myotonia, ptosis, hatchet face, slurred speech, rhinolalia), EMG myotonic pattern, EKG (such as AV-blocks) or routine blood test abnormalities (such as increased CK values or hypogamma-globulinemia) and history of cataract. Its confirmation can come by DNA analysis. At present, only symptomatic therapy is possible and is addressed at correcting hormonal and glycemic balance, removing cataract, preventing respiratory failure and, above all, major cardiac disturbances. Efficacious therapies targeted at the pathogenic mechanism of DM1 are not yet available, while studies that seek to block toxic RNA intranuclear storage with specific molecules are still ongoing.

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INTRODUCTION

Nosography of Myotonic Dystrophies

Myotonic Dystrophies (DM) represent a heterogeneous family of disturbances of muscular fibre release. Such heterogeneity is both genotypic and phenotypic. The predominant clinical aspect is the myotonic phenomenon, which is an abnormal contraction of the muscle fibre after either voluntary activation, hammer percussion (percussion myotonia), or electric stimulation (electric myotonia).

The history of DM begins in the early 1900s, when a German internist, Hans Gustav Wilhelm Steinert (1875-1911, Fig. 1), described a neuromuscular disorder characterized by dystrophic progression with myotonia at clinical examination, for the first time in 1909 (*Über das klinische und anatomische Bild des Muskel schwunds der Myotoniker*). Since then, such syndromic picture has been named 'Steinert's Disease'. Afterwards, Curschmann, Batten and Rossolimo separately described this unusual condition after

Figure 1. Hans Steinert (1875-1911). Reproduced with permission from the Leipzig University archive (H. Steinberg, A. Wagner. Hans Steinert: Zum 100. Jahrestag der Erstbeschreibung der myotonen Dystrophie. Nervenarzt 2008;79:961-970).

Steinert's original article and for this reason the disease is nowadays known as 'Steinert's Myotonic Dystrophy' or 'Steinert's disease', 'Curschmann-Batten-Steinert's syndrome', 'Myotonic Dystrophy', or 'Rossolimo-Curschmann-Batten-Steinert's syndrome', 'Myotonia Atrophica or Dystrophica'.

One major clinical characteristic is 'pleiotropism', which is the involvement of several organs and systems and an autosomal dominant inheritance, which is characterized by an earlier, more severe onset of symptoms in offspring (anticipation phenomenon).

Genetic diagnosis has been available since 1992, when Brook et al demonstrated an expanded CTG-triplet on the 3′UTR noncoding region of the 'Dystrophia Myotonica Protein Kinase' (DMPK) gene on chromosome 19q, in position 13.3 (19q13.3). This nucleotidic repeat is responsible for the disease and its clinical features.[2]

A likely phenotypic and genotypic heterogeneity of myotonic dystrophy was first suggested by Thornton et al in 1994, when he described cases characterized by a myotonic '*Steinert-like*' syndrome with systemic involvement and AD transmission, in which it was not possible to detect the presence of the $[CTG]_n$ expansion on chromosome 19q.[5]

Several descriptions of single or familial cases, characterized by a myotonic syndrome with multisystemic involvement and AD transmission, appeared in the early 1990s, some with mostly distal neuromuscular involvement (*Steinert-like*) and others with proximal involvement (PROximal Myotonic Myopathy 'PROMM'; Proximal Myotonic Dystrophy 'PDM').[6,7]

The finding of such pheno/genotypic heterogeneity raised the problem of how to classify DM, separating common forms (DM1, *Steinert-like*, molecularly determined) from atypical forms of DM (DM2/PROMM and PDM, not molecularly determined). Ranum et al found a link on chromosome 3q for DM2 in 1988, while Liquori et al discovered that the nucleotidic quadruplet $[CCTG]_n$, located on chromosome 3q in position 21 (3q21), in the first intron of the *zinc-finger-protein gene* (ZNF9 gene), was responsible for a large part of the *Steinert-like* or *non-Steinert-like* syndromes without a *link* on chromosome 19q and therefore not due to the nucleotidic $[CTG]_n$ expansion.[3]

The aim of the second IDMC conference (International Myotonic Dystrophy Consortium), held in 1999, was aimed to reset the previous taxonomy of myotonic dystrophies. It was established that the term 'DM2' should be adopted for all progressive multiorgan disorders linked to the DM2 locus.[8] The actual systematization of DM is presented in Table 1.

Table 1. The genotype-phenotype spectrum of myotonic dystrophies

	Genotype		Phenotype
	Chromosome	Expansion	
DM1	19q13.3	CTG	Myotonic dystrophy *(mostly distal)*
DM2 PROMM PDM	3q21	CCTG	Myotonic dystrophy *(mostly distal)* Proximal myotonic myopathy *(mostly proximal)* Proximal myotonic dystrophy *(mostly proximal)*

CLINICAL FEATURES

Steinert's Myotonic Dystrophy (DM1)

Myotonic Dystrophy Type 1 is the most frequent form of myotonic dystrophy, with an estimated minimum prevalence rate of 8-10 (9.31) affected people (up to 12, in some case series) per 100,000 population. It is characterized by autosomal-dominant (AD) inheritance with anticipation phenomenon (earlier and more severe involvement in offspring).

The disease onset in DM1 is extremely variable, but at least 4 subgroups of patients can be distinguished according to onset the: (1) Congenital Form (only maternal transmission of the expanded allele); (2) Juvenile onset; (3) Adult onset; (4) Late onset. In an asymptomatic proband with a pedigree positive for DM1, a definite diagnosis can only be made by DNA analysis, except in cases of obligate carriers.[1]

DM1 is determined by $[CTG]_n$ triplet expansion on chromosome 19q13.3, at the genomic locus of a serin-threonin kinase named DMPK[2] (Fig. 2).

DM1 patients are arbitrarily subclassified on the grounds of $[CTG]_n$ triplet expansion size:

E_1 = 50-149 CTG; **E_2** = 150-1,000 CTG; **E_3** = >1,000 CTG.[8]

There are at least 2 different subclassifications for E_1, E_2, E_3: The choice of adopting one rather than another, depends on the genetic-lab protocol. Another classification also includes an E_4 class, for cases with $[CTG]_n$ >1,500.

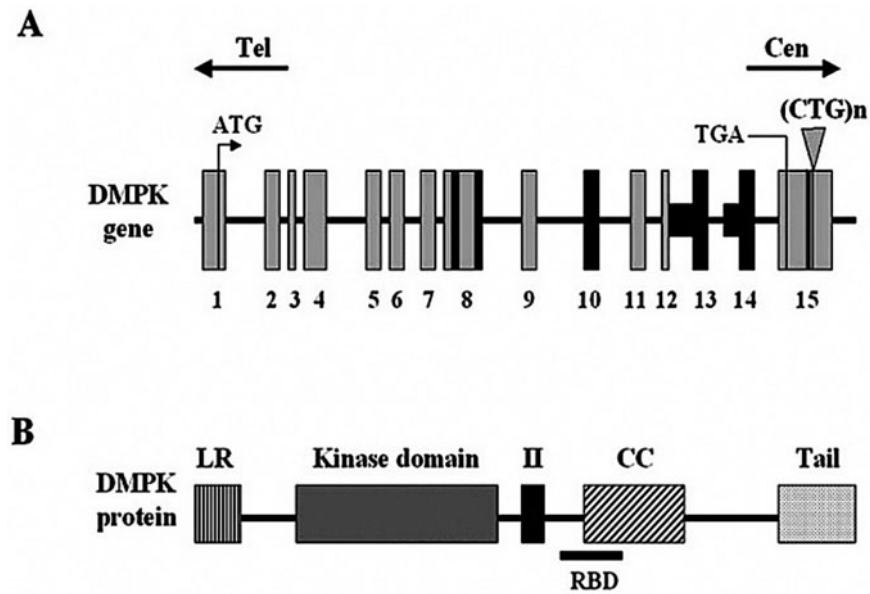

Figure 2. A) DMPK gene. B) DMPK protein. LR: leucine rich region; Kinase domain; II: substrate-specificity site; RBD: possibile rho-binding domain; CC: '*coiled-coil*'; subcellular localization domain. (Modified from: Groenen PJ et al. Constitutive and regulated modes of splicing produce six major myotonic dystrophy protein kinase (DMPK) isoforms with distinct properties. Hum Mol Genet 2000; 9(4):605-16; by permission of Oxford University Press.)

Expansions ranging from 37 to 49 $[CTG]_n$ are considered 'premutations' by some authors, since they are not sufficient to cause the clinical picture, but may be responsible for possible expanding triplets in offspring, given the peculiar instability of the $[CTG]_n$ polynucleotide.[4]

The phenotypic pleiotropic characteristics of DM1 can be summarized as follows:

1. hypotrophic muscular masses of the four limbs, with a disto>proximal distribution; weakness, with or without grip myotonia or percussion myotonia;
2. triangle-shaped or hatchet face (hypotrophy of massetere and temporal muscles);
3. blepharoptosis, mono or bilateral and/or myopathic shape of the mouth (facial weakness);
4. frontal balding;
5. slurred speech, rhinolaly;
6. respiratory failure;
7. cardiac abnormalities or arrhythmia;
8. opacity of the lens, cataract;
9. hypogonadism, diabetes and other endocrine disturbances;
10. osteoskeletal abnormalities;
11. cognitive involvement;
12. daily somnolence, hypersomnia;
13. gastroenteric disturbances.

The characteristic facial phenotype in two DM1 siblings is shown in Figure 3.

Figure 4 shows the anticipation phenomenon.

An over-expanded triplet (usually >1,000 $[CTG]_n$) with early manifestation of symptoms at birth, respiratory failure and severe hypotonia (*floppy baby*) is known as 'congenital myotonic dystrophy' (DM1). Congenital myotonic dystrophy has only been documented in DM1[1,4,9,10] (Fig. 5).

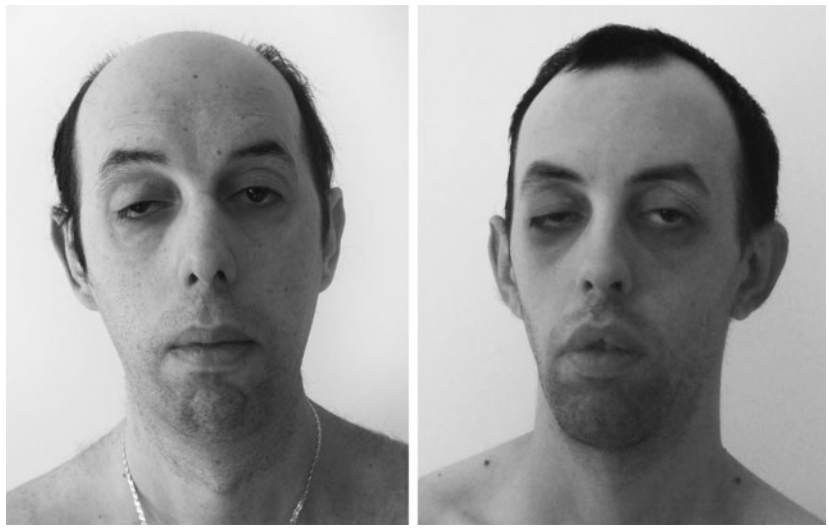

Figure 3. Two siblings affected by DM1. Both present a deficit in hypophyseal secretion of h-GH hormone, tested by GH-RH plus Arginine test.

Figure 4. Woman, 45 y.o. (left), affected by myotonic dystrophy Type 1 (adult onset) and her son, 17 y.o. (right), also affected, with juvenile onset (anticipation phenomenon).

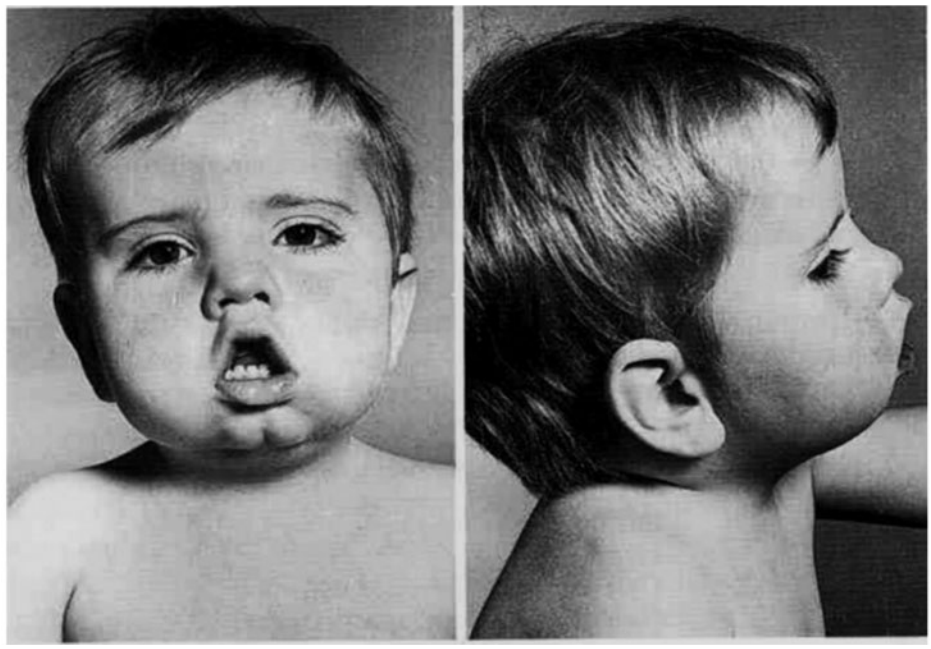

Figure 5. A case of congenital myotonic dystrophy Type 1 is shown. (Reproduced from: Peter S. Harper. Myotonic Dystrophy, 2001:figure 9; ©2001 with permission from Elsevier).

Laboratory and instrumental clinical examinations, aimed at diagnosing a patient suspected as having DM1, involve: routine blood test, electromyography, slit lamp study of the lens and muscular biopsy.

Routine blood test generally reveals hyperCKemia (rarely >1,000 UI/L), suggestive for myopathic disturbances; hypo-γ-globulinemia is not infrequently seen at serum electrophoresis.

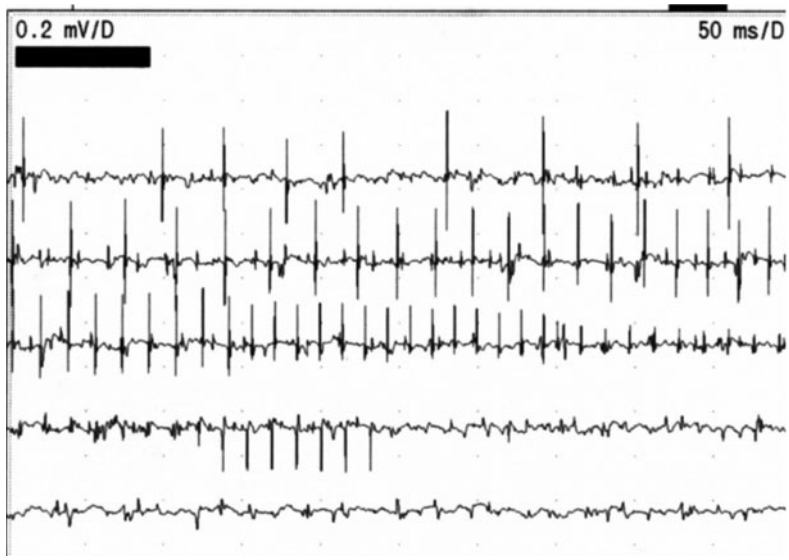

Figure 6. EMG pattern of myotonic discharge.

Electromyography shows peculiar spontaneous electrical insertional activity within the relaxing muscle, explored by electrode-needle, generally diffuse, but prominent in the small muscles of the hand and anterolateral region of the leg. Such activity is commonly referred to as 'electromyographic myotonia' and appears as multiple myotonic discharges known as '*dive bomber potentials*', which are variable for amplitude and frequency, within the single discharge (Fig. 6).

A slit lamp study documents posterior subcapsular iridescent opacities of the lens in many cases, which is very typical for DM1.

Muscle pathology findings are not specific and not pathognomonic. They are extensively described in a dedicated paragraph (see below).

In DM patients, in-depth clinical[11] and instrumental evaluation of cardiac, respiratory, neuropsychological and brain perfusion patterns are mandatory:

1. cardiologic examination (EKG; EKG dynamic-Holter; echocardiography);
2. respiratory investigation (spirometry);
3. neuroimaging (MRI, SPECT);
4. extensive neuropsychological evaluation.

Heart[1,13]

Cardiac involvement in DM1 was firstly described in 1911. Subsequently, several reports documented impaired cardiac conduction and hypotension. In addition, a wide disproportion was observed between symptomatic (16%) and asymptomatic patients with abnormalities on heart investigation. Therefore, an extensive cardiologic study is always suggested to document any anomalies of heart conduction that occur especially in the atrio-ventricular tract (generally AV conduction blocks of first degree) or branch blocks (RBB, LBB) or more complex cardiac conduction defects (at least 80% of

patients). Morphofunctional abnormalities (dilatative cardiomyopathy) are rarely observed (in contrast to some other neurodegenerative or neuromuscular disorders) but, if present, they have to be seriously suspected as a potential source of major arrhythmias (atrial flutter or fibrillation, ectopic beats, sustained atrial or ventricular tachycardia). In these cases, any fibrotic change in the left or right ventricle deserves special attention, especially when associated with a dilatation. Cases of sudden death in young patients affected by DM1 have also been described (muscular performance in these patients is not a good predictor!), while the cause of death in adults and elderly is generally due to pre-existent known or unknown cardiac conduction defects.

Hence, periodic cardiologic follow-up in DM1 patients is mandatory. It should encompass standard EKG, Holter dynamic-EKG and echocardiogram. Moreover, more in-depth cardiologic studies are recommended, such as intracardiac conduction studies, in cases of progression of heart rhythm disturbances, persistence of abnormalities or critical symptomatic patients.

Heart pathology studies suggest a deterioration within the heart conduction system at any level, with prominent fatty and fibrotic changes and myocyte hypertrophy.

An unclear correlation with $[CTG]_n$ expansion has been found, although a trend of increasing risk of heart problems with increasing triplet size is likely. The typical somatic mosaicism demonstrated in several tissues of DM1 patients could reasonably explain these appearently contradictory results. Besides, familiarity for heart conduction defects apart from those due to DM1, could influence the prognosis of this latter (modifying genes theory). The frequency of coronary artery disease in DM1 is not greater than in general population expected of the same age.

Lung[1]

Respiratory problems may also occur in DM1, usually due to severe muscular atrophy, weakness, or skeletal deformity. Besides, it is still debated whether the changes observed in ventilation are mainly due to brainstem dysfunction, peripheral nerve or chemoceptor damage, or by chest and pharyngeal muscle myotonia and/or atrophy. Breathing problems become fairly frequent in the elderly, in whom they may cause severe complications and lead to a weak prognosis in about 40% of patients.

The principal mechanisms through which respiratory problems may occur are: (1) respiratory muscle atrophy/weakness/myotonia with or without diaphragm involvement (restrictive non-obstructive respiratory pattern), particularly important in infancy; (2) central factors, possibly responsible for alveolar hypoventilation, hypercapnia and hypoxaemia could also trigger hypersomnia (whose complex pathogenesis is, however, still unclear. In fact, many central factors seem to combine to determine daily somnolence, such as brainstem, hypothalamic or pituitary dysfunctions); (3) aspiration of gastric material into the bronchial tree, due to insufficient peristalsis or inefficient cardias contraction.

Brain[1,14,15]

The first report of mental disturbances was made in 1937.[27] A poor negative correlation was found between the calculated I.Q. and the $[CTG]_n$ triplet expansion, since $[CTG]_n$ is not a reliable predictor of I.Q. Avoidant personality disorder (uncommon in the general population), with obsessive-compulsive disorder, passive-aggressive and schizotypic traits, not justified by concurrent neuromuscular impairment, has also been described.[14,15]

Neuroimaging studies in DM concern both morphological (by MRI), perfusion (by SPECT) and metabolic aspects (by PET) of CNS.[14] An association between degree of cortical atrophy (MRI) and severity of intellectual impairment, which is more related to other morphological anomalies (thickening of the skull, focal lesions of the white matter, commonly seen in the temporal poles), has not been demonstrated. Perfusion studies mostly show frontal and associative temporo-parietal hypoperfusion, with major brain damage in congenital forms. The presence of white matter hyperintense lesions (WMHLs) and cortical atrophy and dilatation of ventricular spaces has been detected in DM1 patients by brain MRI. In addition, recurrence, localization, diffusion, morphology and relationship with other features of the disease appear quite controversial in several studies.[14] Cortical atrophy in DM1 is a common finding too. However, the distribution and degree of cortical atrophy do not always correlate with cognitive involvement, age at onset, disease duration, neuromuscular status and genetic condition. Only one SPECT study has demonstrated significantly low cerebral blood flow (CBF) in DM1, compared to controls.[14] The major regional-CBF defects were found in both frontal and temporo-parietal regions and more severe degrees of hypoperfusion were seen in maternally inherited DM1. Three PET studies have reported a reduction in the cortical glucose utilization rate (CMRGlu) in DM1, in a CTG-dependent manner.[14]

Brain pathology of post-mortem brains of DM1-patients shows cell loss in specific areas such as in the dorsal raphe nucleus, superior central nucleus, dorsal and ventral medullary nuclei and subtrigeminal medullary nucleus.[1] Neuronal loss in the superficial layer of the frontal, parietal and occipital cortex, as well as in the substantia nigra and locus coeruleus, have also been reported.[1] Neuronal eosinophilic inclusion bodies were described in early studies in up to 30% of the thalamic nuclei of DM1 patients.[1] Their clinical significance is still unclear. The substantia nigra and caudate nucleus may be also involved. These inclusions are made of ubiquitin and microtubule-associated proteins, which means a possible neuropathological substrate for including myotonic dystrophies among the degenerative disorders.[1] Mutant RNA accumulates as nuclear *foci* in specific brain areas where muscleblind proteins are also sequestered, leading to deregulated alternative splicing in neurons of several proteins. RNA *foci* were also detected in the subcortical white matter and corpus callosum. Neurofibrillary tangles of the Alzheimer-type have been demonstrated in DM1.[1,15] Whether the effects of a possible spliceopathy on tau transcripts alone account for the neurodegenerative aspects of patients with DM1 requires further in-depth molecular studies.

Eye[1]

Most frequent abnormality encountered in myotonic dystrophy is cataract, which can be the only manifestation of the disease, especially in elderly patients. It was first described in the early 1900s, but a familial transmission of cataract and muscle disturbances was not documented until in 1918, by Fleischer.[1] The co-existence of cataract and myotonia is suggestive but not diagnostic of DM1. On slit lamp examination early cataract appears as iridescent, multiple, dust-like opacity, usually in the posterior subcapsular layers of the lens. Moreover, the appearance of the cataract can vary in terms of symmetry, lens distribution, iridescence, density and stage of maturation. Several studies indicate that over 80% of DM1 patients, although asymptomatic, have lens abnormalities.[1] The occurrence of cataract in DM1 increases with age, as does specificity. A mature DM1-cataract cannot be distinguished from other cataracts. It should be surgically removed and replaced with

a synthetic lens (about 15% require replacement). Intervention risk and outcome seem to be similar to those of non-DM1 population. The relationship between cataract and neuromuscular status is not strict, since subjects with good muscle performance can have cataract (especially in older age) and severely impaired patients may have no opacities. No clear correlation with diabetes is documented.

Various other disturbances can also occur. Blepharoptosis is susceptible of surgical treatment when the eyelid is dramatically dropping, consequently causing abnormal neck and head posture, with visual loss (personal experience of Author). In these cases blepharoplasty is needed. Lagophthalm or inability to blink is relatively frequent and can determine persistent conjunctivitis and epiphora, with or without keratitis. It has to be properly treated in order to prevent abrasion of the sclera or cornea. Myotonia of the orbicularis oculi or forehead muscles can be occasionally seen in DM1, but blepharospasm is not essential. Although some clinical evidence exists, extraocular muscles are usually not severely involved.[1] Patients quite rarely complain of problems with gaze, such as diplopia.

Glands[1,16-18]

Endocrine abnormalities are frequently found in DM1. The aim of most studies has been to document functional abnormalities in the testes, ovaries, thyroid, parathyroid, pancreas, pituitary and adrenal glands. To date, anecdotic description of single or complex involvement of these glands can be found in literature. However, the main endocrine disturbances concern insulin resistance/diabetes or gonadal functions.

The first description of diabetes mellitus in DM1 dates back to 1950. Since then, several studies have been conducted to test glucidic metabolism in DM1 patients, who seem to have a four-fold risk of developing diabetes compared to healthy controls. Globally, diabetes does not frequently occur in DM1, while the finding of glucose intolerance or insulin resistance with persistently elevated insulin plasma levels after glucose load is common, even in the case of normal blood glucose titres (over 60%). A convincing explanation has been given by the recent demonstration of atypically distributed insulin receptors on the myofibre surface, likely due to an abnormal mRNA splicing process. In DM1, the pattern of insulin resistance is more common than diabetes.

The presence of testicular atrophy has been very well documented since the first descriptions of myotonic dystrophy. Here, macro and microscopic pathologic changes are frequently associated with impaired spermatogenesis, which often leads to oligo-azoospermia or adynamia of sperms. Impotence is sometimes observed, but may be underestimated and might be due to secondary smooth muscle or gonadal dysfunction. In women, gonadal abnormalities are frequently seen and less specific. DM1 females usually complain of irregular menstruation, but the high prevalence of gynaecological symptoms in healthy controls does not permit to generalize this observation to the entire DM1 population. Parallel to primary gonadal insufficiency, follicle-stimulating hormone (FSH) is usually found to be increased in both men and women, while luteinizing hormone (LH) is mostly normal. Infertility or reduced reproductive loss is frequent. During pregnancy, some major complications have been observed significantly more often in DM1 than in the general population, such as hydramnios (15 to 30%), low foetal maturity, neonatal deaths, retained placenta or placenta praevia and the need for caesarean delivery in about 10%.

The involvement of the pituitary gland in DM1 is not well documented. Most common abnormality encountered is an increase of FSH hormone in cases of primary hypogonadism.[1] Besides, there is no consensus about the modality of h-GH secretion.

Early evidence of enlarged cranial bones (skull thickness with parasinusal hypertrophy) seemed to suggest that an excessive increase of growth hormone (GH) could be responsible, such as in acromegaly. More recent observations suggest that impaired GH secretion after proper stimulation (as GH-RH and arginin) can be seen in DM1 (about 30%), without somatic evidence of GH defect or hypopituitarism. The relationship between this abnormal GH-curve response to stimulation and insulin resistance is not clear.

Prolactin and thyroid-stimulating hormone (TSH) do not seem to significantly differ from the general population, while dehydroepiandrosterone (DHEAs) is frequently found below normal levels in up to 80% of the DM1 cases.

Gastroenteric[1]

Smooth muscle can be involved in DM1 as can skeletal muscle. Its involvement—usually of mild severity—is not necessarily consistent with striated muscle. Gastrointestinal tract (including gall bladder) and urinary pathways may be involved.

Gastrointestinal symptoms are frequently reported by patients, who usually complain of several disturbances, with variable frequency, occurrence and severity (dysphagia, vomiting, abdominal pain, constipation, diarrhoea, incontinence).

One of the most frequently reported problems is associated with pharyngeal and oesophageal functions. It is usually characterized by difficulty in swallowing, irrespective of the severity of neuromuscular involvement or disease duration/onset. The nature of this problem seems to have multiple causes. Failure or changes in peristalsis, myotonia with abnormal contraction-decontraction times, enlargement of the oesophagus, the major consequence of which may be the aspiration of material into the bronchial tree, frequently associated with severe complications or death, especially in those patients who also present respiratory restrictive insufficiency.

Malabsorption, secondary to small bowel disturbances, appears rather anecdotal and negligible. Conversely, abdominal pain is quite common, particularly in young patients. The most typical features observed are those of 'irritable bowel syndrome'. They are usually mild and generally do not need surgical intervention, and should always be taken seriously to avoid surgical or anaesthesiological complications. Cases of colonic pseudo-obstructions are described, but rare.

Abnormal behaviour in the contraction-relaxation responses of internal and external anal sphincters has also been described. Also increased values of serum bilirubin and gamma-GT have been reported, in addition to cholelithiasis (significantly higher than in the general population), to be possible signs of cholestasis, likely due to an abnormal gall bladder emptying mechanism or abnormalities in bile acid metabolism. Mini-invasive surgical procedures for cholecistectomy should be carried out when necessary.

MUSCLE PATHOLOGY[19,20]

The presence of abnormalities in muscle pathology in DM1 is typical but not pathognomonic (Fig. 7).

- Centralized and/or internalized nuclei: They can be seen even at an early stage of the disease. Usually, the greater the number of internalized nuclei, the greater the muscular involvement of the patient. In longitudinal sections, typical

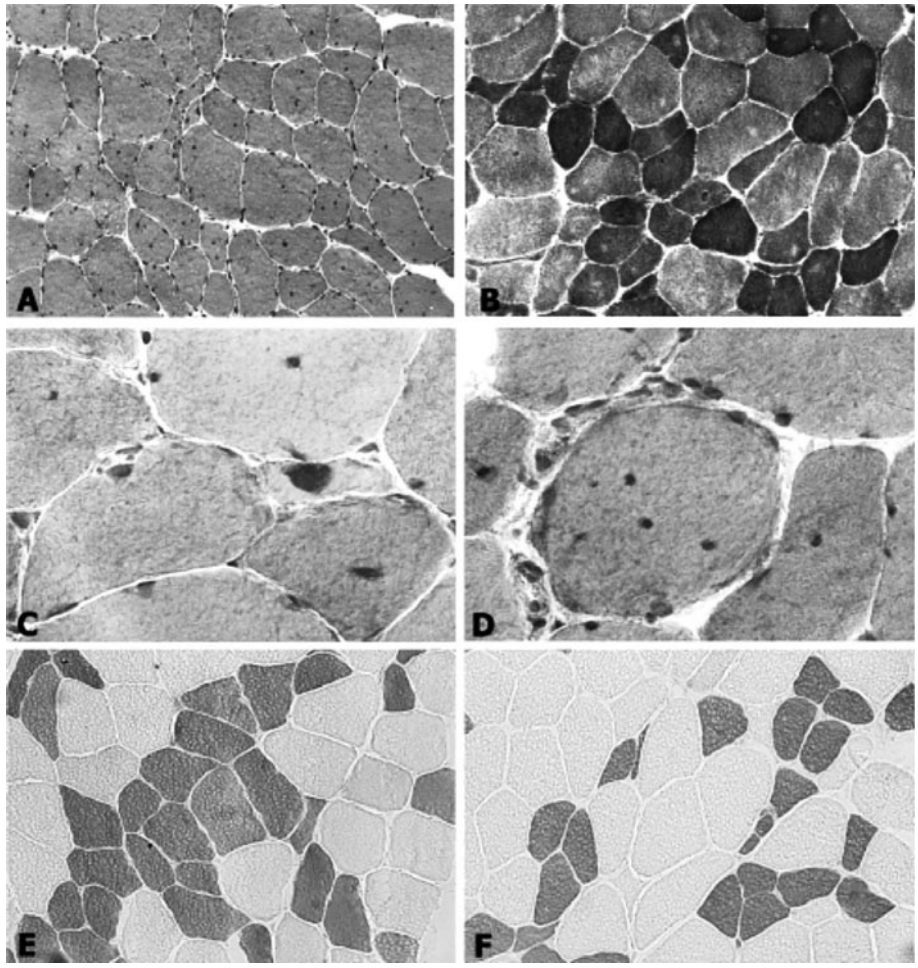

Figure 7. DM1 muscle pathological findings. Transverse section of voluntary muscle fibres, seen on optical microscopy. A) Haematoxylin-eosin (×10): internalization of nuclei, with fibre polydimensionalism. B) NADH-TR (×10): scattered central nuclei, along with Type 1 fibre hypotrophy and moth-eaten, scarcely reacting fibres. C) Gomori trichrome (×40): sarcoplasmic masses, central nuclei, atrophic fibres. D) Gomori trichrome (×40): single fibre with multiple internalized nuclei and sarcoplasmic masses, resembling a ring-fibre. E) Acid ATPase (×10): homotypic Type 1 fibre-grouping. F) Acid ATPase (×10): Type 1 fibre atrophy with Type 2 fibre hypertrophy.

chain-distributions can be observed, each of which can contain up to 20 nuclei. At present, such phenomenon may not be exclusively due to nuclear division; nuclear migration along the muscle fibre could be responsible for this pathological finding. Moreover, the presence of morphological heterogeneity of the nuclei has been reported: Some are picnotic, others appear pale and enlarged.

- Ring-fibres: Fibres with ring-shape myofibrils, first described by Heidenhain in 1918 and subsequently confirmed by Dubowitz and Brooke in 70% of biopsies, correlated with chronicization of the disease.

- Fibre-polydimensionalism: Type 1 and Type 2 fibres are clearly dishomogeneously distributed, with the former having typical small diameter. Such discrepancy evolves to marked atrophy of Type 1 fibres, whereas Type 2 fibres can occasionally hypertrophize. This association seems to be very specific for DM1, since other muscular dystrophies or other myotonic disorders do not usually have similar pictures.
- Sarcoplasmic masses can co-exist in homogeneous sarcoplasmic areas. They are frequently seen close to ring-fibres. Histochemical analyses conducted by Engel in 1962 showed that they are made of dysorganized intermyofibrillar material, where myofibrils and associated enzymes are completely absent. In 1970 Mussini et al clarified the regenerating nature of the masses, by ultrastructural microscopy.
- Several other myopathic phenomena, such as connective tissue proliferation, can be documented. In an advanced stage of the disease, angulated fibres with degeneration-regeneration aspects (signs of necrosis, basophilic fibres, phagocytosis, fibrosis, lobulated and moth-eaten fibres) can be occasionally detected. (Harper 2001, Dubowitz 2007, Mussini 1970).

PATHOGENESIS OF DM[21,22]

DM1 and DM2 are the only human genetic autosomal dominant inherited neuromuscular disorders with multi-system effect, in which the disease phenotype has been directly linked to disrupted regulation of alternative splicing, due to abnormal accumulation of toxic RNA within the affected nuclei.[22] To date, the pathogenesis of both DM has not yet been completely understood. The deposition of an abnormal transcribed and nontranslated RNA from the sequence $[CTG]_n$ at DMPK-gene *locus*, within the nuclei of affected cells that express this gene, is supposed to be responsible for the *primum movens* of the pathogenic chain. The demonstrated epiphenomenon of this process is the presence of intranuclear '*foci*' of RNA, pathologically deposited inside the nuclei of affected cells (Fig. 8).

In the beginning the description of intranuclear '*foci*' of *CTG-repeat* transcripts temporarily went unnoticed and only at a later stage received greater interest. This shows that RNA can acquire, through an aberrant deposition process, an effect of '*toxic gain-of-function*'. This would justify the mutual complexity and similarity of syndromic features of both DM1 and DM2, although the genes recognized as responsible for the two diseases are very far from each other on the genetic map and the proteins which these two genes encode for are so functionally different.

However, in the past at least two different pathogenic mechanisms had been hypothesized to explain the pathogenesis of DM1: aploinsufficiency of DMPK gene and aploinsufficiency of neighbouring genes. In the first case a transcriptional defect of DMPK-gene is enough to determine at least part of the symptoms, related to the reduction of the DMPK transcripts and, consequently, of the protein; documented decreased rates of DMPK-mRNA in the myofibres of the patients seem to support this hypothesis, as does the development of arrhythmogenic cardiopathy in the DMPK-gene *knock-out* mouse model. The second hypothesis (*neighbouring genes* like SIX5, DMWD and some others) could justify the heterogeneous clinical features of DM1.

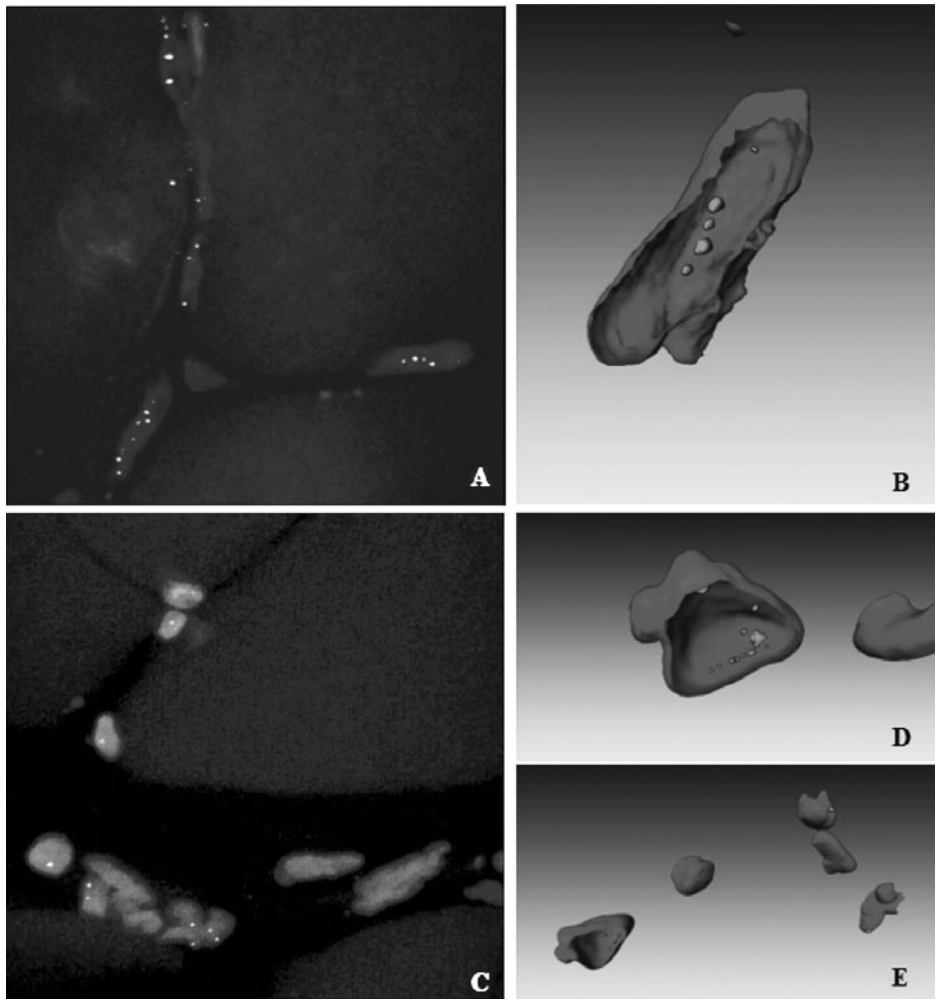

Figure 8. A) *Foci* of pathological accumulation of aberrant RNA (CTG-triplets) are evidenced by the FISH method in a case of DM1. B) A 3D-reconstruction of the detected *foci*, to better describe number, shape and intranuclear localization of the *foci*. C) *Foci* of CCTG-quadriplet repeats are shown and evidenced by FISH in a case of DM2. D,E) 3D reconstructions, in the same case.

At present, the most accredited pathogenic hypothesis for DM1 is that of a *toxic gain-of function of CUG-repeat*. Simultaneously several research studies have developed, on the role of certain endonuclear proteins targeting RNA and involved in endonuclear transfer mechanisms (*trafficking*), post-transcriptional modifications and newly-synthetized RNA catabolism. Among those, investigated are the dsRNA-BPs (double stranded *RNA binding proteins*, like PKR, TAR, RNA helicase A) and, above all, proteins of the MBNL family (muscleblind proteins), particularly MBNL1. The observation of intranuclear '*foci*' of aberrant RNA within the myonuclei and of their

colocalization with aggregates of MBNL, has aroused great interest (data based on the FISH method, Fig. 8). Conversely, an increased intranuclear concentration of dsRNA-BPs and a decreased intranuclear concentration of MBNL has been documented. The hypothesis is that a 'sequestration of MBNL' by aberrant RNA, produced by the CTG triplet expansion, occurs. Therefore, the larger the CTG triplet expansion on chromosome 19q, the stronger the efficacy of the sequestration. In this way, the normal MBNL activity on the healthy RNA would be torn apart by a 'subtractive' mechanism. In this balance, dsRNA-BPs, recalled into the nucleus, would be increased, with a subsequent 'loss of stechiometric balance' of various parts and an impairment in the processing of many other neotranscribed endonuclear RNAs.

Several studies have been aiming to understand how alternative splicing dysregulation can be involved in the pathogenesis of myotonic dystrophy. To date, the results of these studies suggest that it corresponds to the clinical syndromic complexity of DM.

In healthy subjects, primary mRNA transcripts present in normal cells/myoblasts are subjected to post-transcriptional changes by alternative splicing process. This process normally produces different proteic isoforms, specific for that particular cell-line at that particular time. It is likely that in DM1-myoblasts certain primary mRNAs undergo an abnormal splicing process, resulting in aberrant secondary transcripts, unable to produce the original proteic isoform. The new proteic isoform, which can be atypical for that particular cytotype or completely original, would have little or no chance of becoming a functional protein in the cellular context where it has been produced.

This kind of anomaly has been proposed for at least 5 post-transcriptional processes: (1) chloride channel splicing; (2) insulin receptor splicing; (3) cardiac T-troponin splicing; (4) tau-protein splicing; (5) myotubularin splicing.

Each phenotype appears to correspond to specific damage, secondary to the abnormal ribonuclear processes.

In detail: (1) anomaly of the proteic isoform of the chloride channel (ClC-1) is believed to cause myotonia because of changes in the opening-closing kinetics of the voltage-dependent channel; (2) alteration of the insulin receptor is assumed to determine insulin-resistance, because the insulin receptor (IR) is unable to interact with its own ligand (insulin), for conformational reasons; (3) cardiac T-troponin (cTNT) anomaly could be implicated in the development of the arrhythmogenic cardiopathy typical of DM1; (4) abnormalities in tau-protein, associated with microtubules, appear to justify cognitive deficits; (5) myotubularin (*myotubularin-related-1*, MTMR1) deregulation mechanisms are considered to be responsible for the severe congenital phenotype and for its marked muscular atrophy and weakness.

Cataract, gonadal insufficiency and hypo-γ-globulinemia, remain unexplained.

The pathological sequence of these molecular events (deposition of aberrant RNA endonuclear '*foci*', sequestration of MBNL and consequent 'compensatory' increase in other RNA binding proteins and thereafter secondary dysregulations of RNA processing that lead to the formation of unusual, malfunctioning proteic isoforms) could be extended to the entire DM clinical spectrum of polynucleotide repeat-related diseases (Fig. 9).

Hence, although both DM1 and DM2 originate from two different genetic *loci* (chromosomes 19 and 3), they seem to determine complex and surprisingly similar syndromic features, since a 'common final pathway' can be recognized in their pathogenesis, as explained by Day et al[22] (Fig. 10).

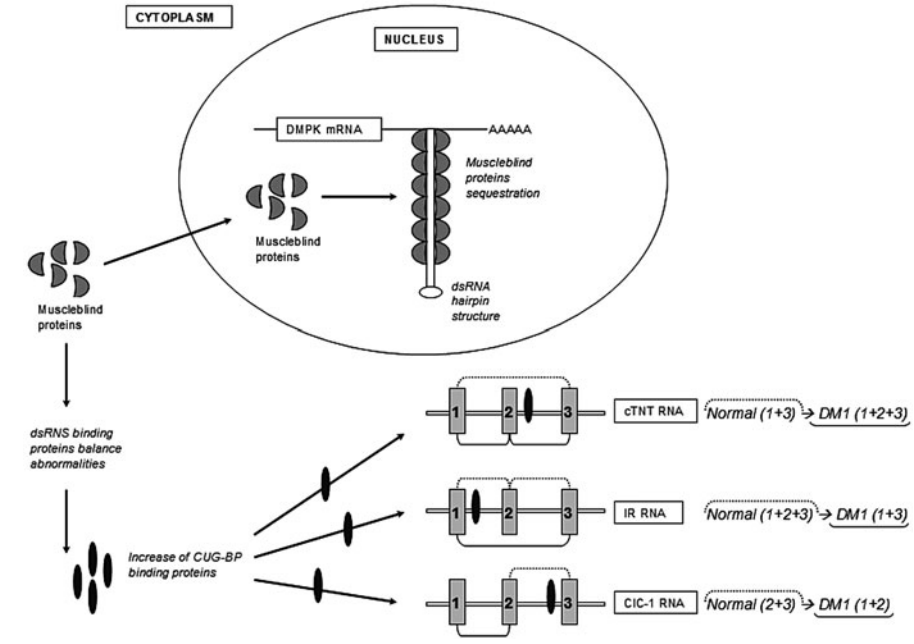

Figure 9. Pathogenic model of DM1: MBNL sequestration and increase in CUG-BP are followed by the alteration in primary mRNA transcript processing, with subsequent development of mature but pathological endonuclear mRNAs, from which several atypical proteic isoforms can derive.

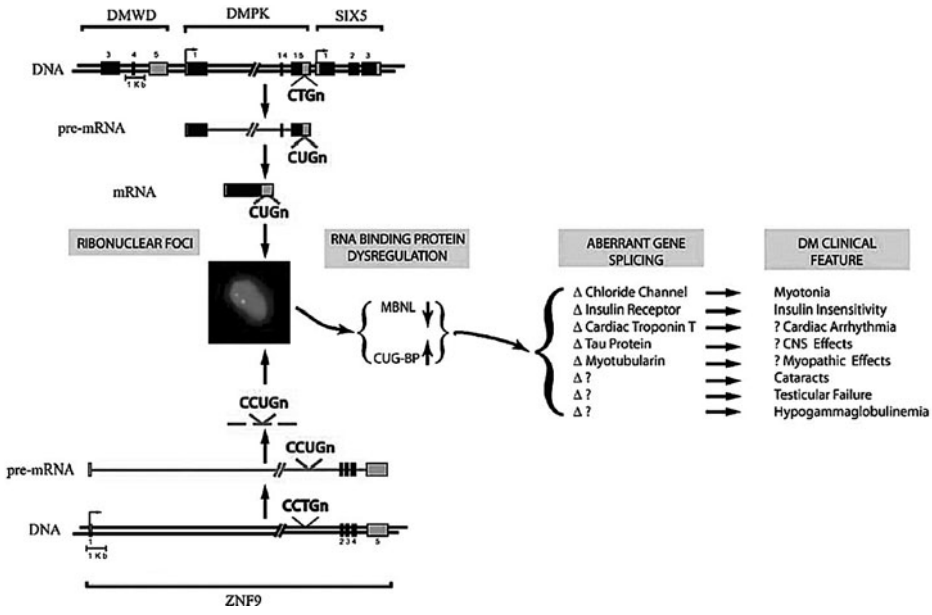

Figure 10. Pathogenesis of DM: two distinct *loci* for a common final pathway. Reproduced from: Day JW, Ranum LP. Neuromusc Disord 2005; 15:5-16;[22] ©2005 with permission from Elsevier.

MANAGEMENT, TREATMENT AND FUTURE PERSPECTIVES

At present, only symptomatic therapies are available for myotonic dystrophy. These treatments are generally safe, useful and their aim is to resolve -partially or completely- many symptoms that can occur in DM1.

Myotonia usually responds to mexiletine (200 to 600 mg/day), whose benefits have been documented in several studies.[23] A major contraindication to high doses of mexiletine is the existence of a heart conduction defect. In case of treatment with mexiletine or any other voltage-gate channels blockers, regular periodic cardiologic follow-up is highly recommended.

The presence of EKG and dynamic-EKG hallmarks of AV-blocks, that worsen over time or become complicated by additional heart disturbances or clinical symptoms, suggests more in-depth cardiologic investigations. Patients with major, potentially harmful, arrhythmic disturbances are candidates for pacemaker implantation. The implantation of an intracardiac defibrillator device must be considered in very selected cases (e.g., symptomatic patients with personal history of ventricular fibrillation).

Diabetes can be controlled by oral drugs or insulin, especially when it cannot be controlled by adequate physical activity or diet adjustment.

Gastrointestinal disturbances should always be recognized, in order to prevent aspiration of swallowed material into the bronchial tree (pharyngo-oesophageal dysfunction) and the consequences of acute colonic pseudoobstruction.[1]

Poor response to oral modafinil treatment has been documented in cases of recurrent daytime somnolence.[28]

Some peculiar conditions deserve a surgical approach. A mature cataract can be surgically removed and blepharoplasty considered in patients with severe blepharoptosis with visual loss. Surgery is also an option for tendon retractions and severe spine deformity, especially in children in association with serious respiratory impairment. In these cases, respiratory insufficiency and heart disturbances must be properly evaluated before any intervention, to avoid complications related to general anaesthesia.[12]

Hormone replacement therapy with testosterone in cases of hypogonadism has proven ineffective,[24] while small populations of DM1 patients have been treated with IGF-1, without significant side effects. Human GH replacement therapies in patients who show reduced h-GH secretion after stimulation, have been proposed, but these studies are still inconclusive.[25,26]

Unfortunately, therapies designed to arrest or invert the process of muscle atrophization are not available. Moreover, techniques capable of restoring a correct genetic condition, reducing the causative $[CTG]_n$ triplet expansion to normal values, have not been developed. However, the progressing knowledge on the pathogenic mechanisms underlying myotonic dystrophies is disclosing new perspectives on the possibility of synthesizing effective drug targeting to precisely interrupt the early pathologic cascade of events that leads from $[CTG]_n$ repeats to aberrant splicings. The principles of RNA interference have been taken into account and studies in this field are ongoing.

CONCLUSION

Myotonic dystrophy is the most frequent autosomal dominant inherited muscular dystrophy. A definite diagnosis is possible only by DNA analysis, since muscle pathology changes are typical but not pathognomonic. DM1 is due to a $[CTG]_n$ triplet expansion on

chromosome 19q. This nucleotide expansion acquires a *toxic gain-of-function* within the affected myonuclei, thus leading to an abnormal RNA splicing process that involves several other genes. The severe congenital picture of myotonic dystrophy Type 1 is possible only in case of maternal transmission to the newborn. Anticipation phenomenon means earlier and severer involvement in offspring. DM1 is characterized by pleiotropism (involvement of several organs and systems) with variable severity. The patients can have major heart disturbances, endocrine involvement with insulin resistance and reduced fertility, cataract, respiratory insufficiency, cognitive impairment, gastroenteric involvement. At present, treating DM1 mostly means adopting symptomatic pharmacological or surgical strategies, or preventing the occurrence of potentially life-threatening cardiac and respiratory problems. It is reasonable to hypothesize that in the future new and safe pharmacological options—targeted to the gene defect or pathogenic mechanism—will be available to cure myotonic dystrophy.

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