# Università degli Studi di Padova

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**Dipartimento di Neuroscienze**

**Scuola di Dottorato di Ricerca**
**in Scienze Mediche Cliniche e Sperimentali**

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## BRAIN INVOLVEMENT IN MYOTONIC DYSTROPHIES

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## ABSTRACT

Objective: to determine the degree of brain involvement in a cohort of DM1 and DM2 patients by brain investigations and functional tests and to compare the results of the two groups.

Background: Myotonic Dystrophies type 1 and type 2 (DM1, DM2) are multisystemic disorders due to polynucleotide expansions. Previous studies on brain involvement by neuroimaging and functional methods led to contradictory results.

Materials and methods: 50 molecularly defined DM1 and 14 DM2, were recruited for the study. Age at recruitment, age at disease onset, disease duration and educational level were recorded. Neuromuscular assessment was done by MIRS. An extensive neuropsychological battery was performed in 48/50 DM1 and in a control group of 44 healthy matched subjects. 46/50 DM1 and 12/14 DM2 underwent brain MRI; 21/50 DM1 and 9/14 DM2 underwent brain perfusion SPECT, with semiquantitative analysis of the results. MRI images were classified by ARWMC (age related white matter changes) score, in order to quantify recurrence, localization, patterns of distribution of white matter hyperintense lesions (WMHLs) in our two cohorts. MRI results were matched to SPECT and to neuropsychological results.

Results: 37/46 DM1 and 10/12 DM2 had abnormal MRI imaging, showing scattered supratentorial, bilateral, symmetrical focal or diffuse WMHLs. A typical temporo-insular diffuse subcortical pattern was seen in DM1 only, with no correlation with cognitive involvement. Major cognitive involvement was seen in the case of diffuse frontal lesions. A relationship with CTG expansion size was documented for DM1.

SPECT showed minimal hypoperfusion in the posterior cortex planes, in DM1 and, to a lesser extent, in DM2. Very mild degrees of involvement in the DM2 cohort were seen. Conclusions: neuroimaging and functional investigations confirmed a more severe involvement of the brain in DM1 compared to DM2. A temporo-insular diffuse lesional pattern, specific for DM1, was found on MRI. This confirms greater expansion size as a risk factor for more extensive brain involvement in DM1.

# INTRODUCTION

## NOSOGRAPHY OF MYOTONIC DYSTROPHIES

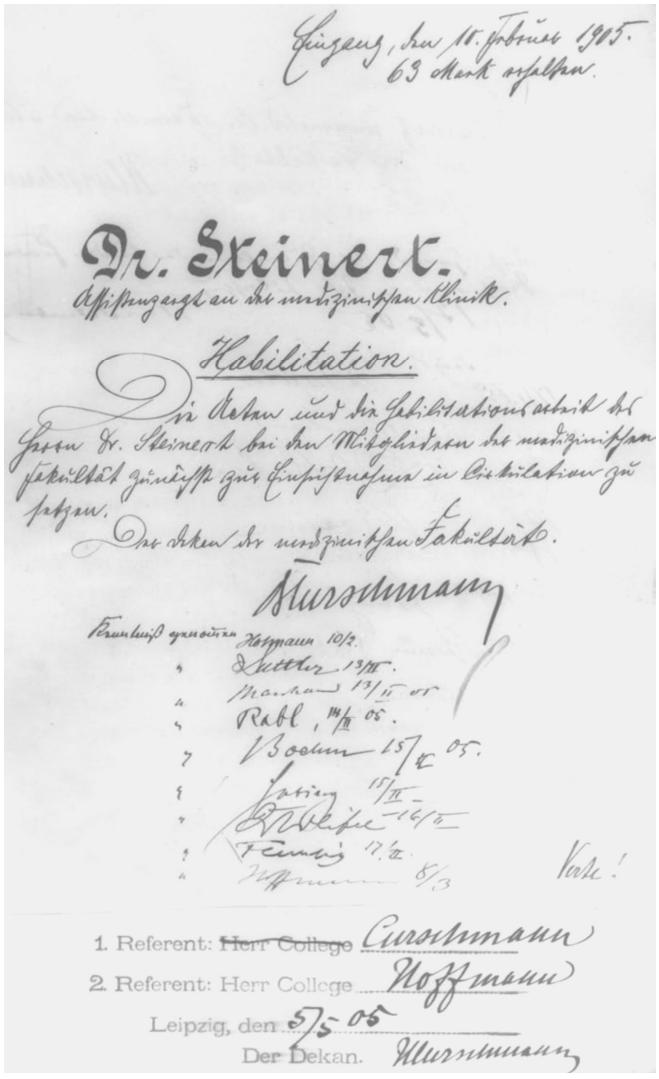
Myotonic Dystrophies (DM) represent a heterogeneous family of disturbances of muscular fibre release. Such heterogeneity is both genotypic and phenotypic.

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 nosography of DM begins in the early 1900, when a German Internist, Hans Gustav Wilhelm Steinert (1875-1911, Figg. 1 and 2), exactly in 1909, for the first time described a neuromuscular disorder characterized by dystrophic progression with myotonia at clinical examination. (*Über das klinische und anatomische Bild des Muskel schwunds der Myotoniker*). (Steinert 1909, Steinberg 2008)

**Fig. 1.** Hans Steinert (1875–1911). From the Leipzig University archive (H. Steinberg, A. Wagner. *Hans Steinert: Zum 100. Jahrestag der Erstbeschreibung der myotonen Dystrophie.* Nervenarzt 2008;79:961–970).

Since then, such syndromic picture would have been named ‘Steinert’s Disease’. Afterwards, Curschmann and Batten (and Rossolimo himself) mentioned this pathologic condition in separated works, a little later after 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’.


Leipzig, den 10. Februar 1905.
63 Mark erhalten.

Dr. Steinert.
Assistenzarzt an der medizinischen Klinik.

Habilitation.

Der Dekan der medizinischen Fakultät.
Curschmann

Freundlich genehmigt Hoffmann 10/2.
" Seiffer 13/II.
" Marburg 13/II 05.
" Rabl, 14/II 05.
" Bonn 15/II 05.
" Jastrow 15/II.
" Edinger 14/II
" Penzoldt 17/II.
" Hoffmann 8/3

Viele!

1. Referent: ~~Herr College~~ Curschmann
2. Referent: Herr College Hoffmann
Leipzig, den 5/3 05
Der Dekan. Curschmann

**Fig. 2.** Heinrich Curschmann, Dean of the Faculty of Medicine, confers Dr Steinert the habilitation, February 10th, 1905. (Steinberg 2008).

The characteristic EMGraphic finding is represented by the *dive bomber potentials* (Fig. 9).

Other major characteristic, typical of myotonic dystrophies, is the 'pleiotropism', which is the possible involvement of several organs and systems, as manifastation of the disease, and a typical autosomal dominant inheritance, that is characterized by an earlier and severer onset of the symptoms in offsprings (anticipation phenomenon).

Such clinical disturbances represent what has almost exclusively been indicated as Myotonic Dystrophy (DM), at least until the first ninties.

Molecular diagnosis of DM by DNA analysis is provided since 1992, when Brook *et al.* showed an expanded CTG-triplet on the 3'UTR non-coding region of the 'Dystrophia Myotonica Protein Kinase' (DMPK) gene on chromosome 19q in position 13.3 (19q13.3). This nucleotidic repeat is able to determine the development of the disease and of its clinical correlated disturbances. (Brook 1992).

The problem of the phenotypic and genotypic heterogeneity of myotonic dystrophies was firstly suggested by Thornton *et al.* in 1994 with the description of clinical cases characterized by a myotonic '*Steinert-like*' syndrome with systemic involvement and familial transmission of the autosomal-dominant kind (AD), where it was not possible to detect the presence of the CTG expansion on chromosome 19q. (Thornton 1994).

Several descriptions of single or familial cases characterized by myotonic syndrome with multisystemic involvement and autosomal dominant transmission took place in the first nineties, in some cases with mostly distal neuromuscular involvement

(*Steinert-like*), and in some cases with proximal involvement (PROximal Myotonic Myopathy 'PROMM'; Proximal Myotonic Dystrophy 'PDM'). (Ricker 1994).

The finding of such pheno/genotypic variants evidenced the serious problem of the 'subclassification' of DM, so that it was necessary to distinguish typical forms of DM (DM1, *Steinert-like*, molecularly determined) from atypical forms of DM (DM2/PROMM and PDM, not molecularly determined).

Parallely to the increasing interest by clinical myologists in attempting to better classify such extreme phenotypical heterogeneity, the genetists were stimulated too, in researching those chromosomic anomalies that could properly justify the percentage of myotonic dystrophies without any CTG triplet expansion on chromosome 19q and therefore indicated as 'orphan disorders'.

Ranum *et al.* found a link on chromosome 3q for DM2 in 1988, while Liquori *et al.* discovered that the nucleotidic quadriplet (or tetraplet) CCTG located on chromosome 3q in position 21 (3q21) sited in the first intron of the *zinc-finger-protein gene* (ZNF9 gene) is responsible of both the etiopathogenesis and of the transmission of a large part of the *Steinert-like* or *non-Steinert-like* syndromes without a *link* on chromosome 19q and therefore not due to the well known nucleotidic CTG expansion.

The second IDMC conference (International Myotonic Dystrophy Consortium), kept in 1999, was aimed at re-setting the previous taxonomy of myotonic dystrophies, re-organizing the results of all previous clinical and basic research works. It was established that the term 'DM2' should be adopted for all progressive multiorgan disorders linked to the DM2 locus (The International Myotonic Dystrophy Consortium, 2000).

More recently, Le Ber *et al.* described a wide pedigree, whose members risulted affected by a myotonic syndrome, AD-transmitted, similar to DM1/DM2, but without any link to chromosomes 19q or 3q, and peculiar for the association with several psychiatric disturbances (fronto-temporal dementia, major depression and other disturbances of affectivity, schizophrenia, oligophrenia,…). The linkage analysis suggested a possibile involvement of chromosome 15q21-24 in such syndromic picture, but further studies excluded any parallelism with DM families. (Le Ber 2004)

The actual systematization of DM is synthetically reported in table 1.

**Tab. 1.** The genotype-phenotype spectrum of myotonic dystrophies.

| **Myotonic Dystrophies** | | | |
|---|---|---|---|
| | Genotype | | Phenotype |
| | Chromosome | Expansion | |
| DM1 | 19q13.3 | CTG | myotonic dystrophy *(mostly distal)* |
| DM2 PROMM PDM | 3q21 | CCTG | myotonic dystrophy *(mostly distal)*<br>proximal myotonic myopathy *(mostly proximal)*<br>proximal myotonic dystrophy *(mostly proximal)* |
| DMn | ? | ? | proximal myotonic myopathic syndrome |

## CLINICAL FEATURES

### *STEINERT'S MYOTONIC DYSTROPHY (DM1)*

Myotonic Dystrophy type 1 represents the most frequent myotonic dystrophy, with an estimated minimum prevalence rate of 8-10 (9.31) affected people (until 12, in some casistics) per 100.000 inhabitants (Siciliano 2001).

It is characterized by autosomal-dominant (AD) inheritance with anticipation phenomenon (earlier and severer involvement in offspring) (Figure 3 and 6).

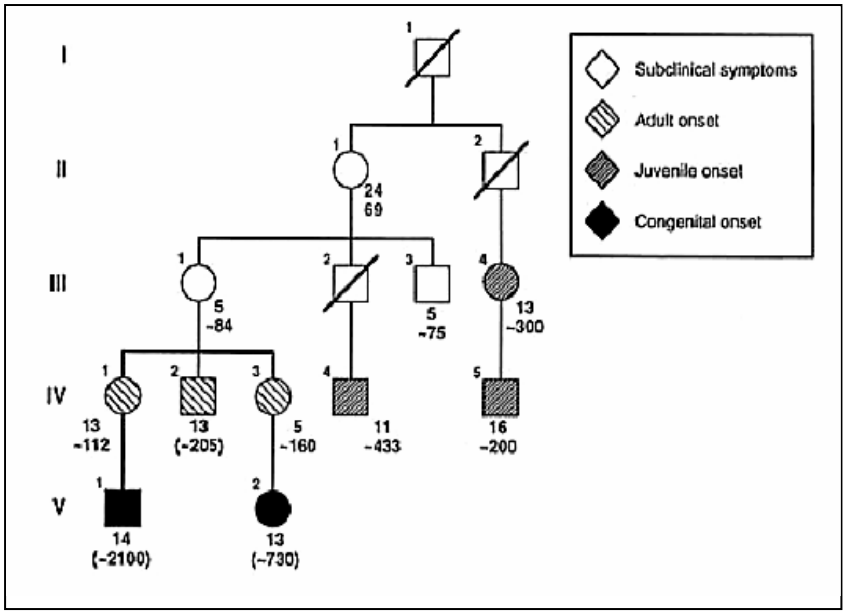



**Fig. 3**. Pedigree indicating anticipation phenomenon.

The disease onset of DM1 is extremely variable, but we can distinguish at least 4 subgroups of patients on the basis of the onset: 1) Congenital Form (only maternal transmission of the expanded allele); 2) Juvenile onset; 3) Adult onset; 3) Late onset. There are almost asymptomatic patients, in whom the diagnosis can be made only by DNA. In these patients, the clinical suspect exclusively comes from a positivity of DM1 within their own pedigree.

DM1 is determined by the CTG triplet expansion sited on chromosome 19q13.3, at the genomic locus of a serin-threonin kinase named DMPK (Fig. 4).

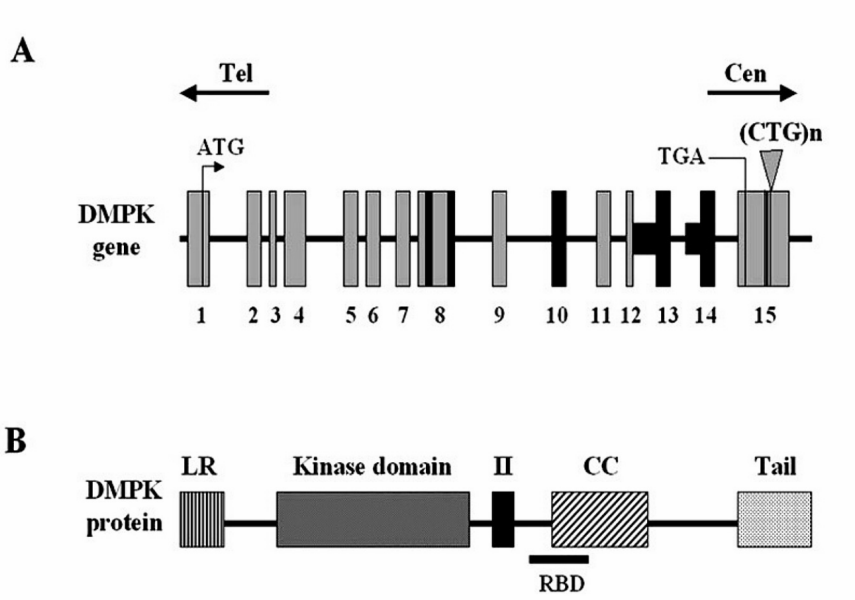



**Fig. 4.** A: DMPK gene. B: DMPK protein. LR: leucine rich region; Kinase domain; II: substrate-specificity site; RBD: possibile rho-binding domain; CC: '*coiled-coil*'; subcellular localization domain. (Modif. from: Groenen GTA, Wansink DG, et al.).

The patients affected by DM1 can even be arbitrariously subclassified on the basis of the CTG triplet expansion size:

**$E_1$** = 37 - 150 CTG; **$E_2$** = 150 - 1000 CTG; **$E_3$** = over 1000 CTG.

It must be specified that at least 2 different subclassification $E_1$, $E_2$, $E_3$ exist: the choice of adopting one, rather than another, depends on the laboratory that performs the genetical analysis; for example, a classification also includes an $E_4$ classification, for cases with CTG>1500; neverthless in the present work we will consider the one reported extensively above, which is the most commonly adopted.

Expansions ranging from 37 to 49 CTG are considered 'premutations' by some Authors, since they are not sufficient to determine the developement of the clinical

picture. However, they can be transmitted to the offspring as well, and can be subjected to amplification because of the peculiar instability of the CTG polynucleotide.

The phenotypic pleiotropic characteristics of DM1 are reassumed as follows:

1) Hypotrophic muscular masses of the four limbs, with a disto>proximal distribution, with relative weakness, associated with occasional grip myotonia or percussion myotonia;
2) triangle-shaped face or hatchet face (hypotrophy of massetere and temporal muscles);
3) blepharoptosis, mono or bilateral, and myopathic mouth (facial weakness);
4) frontal balding;
5) slurred speech;
6) rhinolaly;
7) opacity of the lens;
8) hypogonadism, diabetes and other endocrine disturbances;
9) osteoscheletal abnormalities;
10) cognitive involvement;
11) daily somnolence; hypersomnia
12) gastroenteric disturbances.

The characteristic facial phenotype of DM1 is shown in Fig. 5.

The following figure (Fig. 6) shows how the anticipation phenomenon, as it clinically displays.

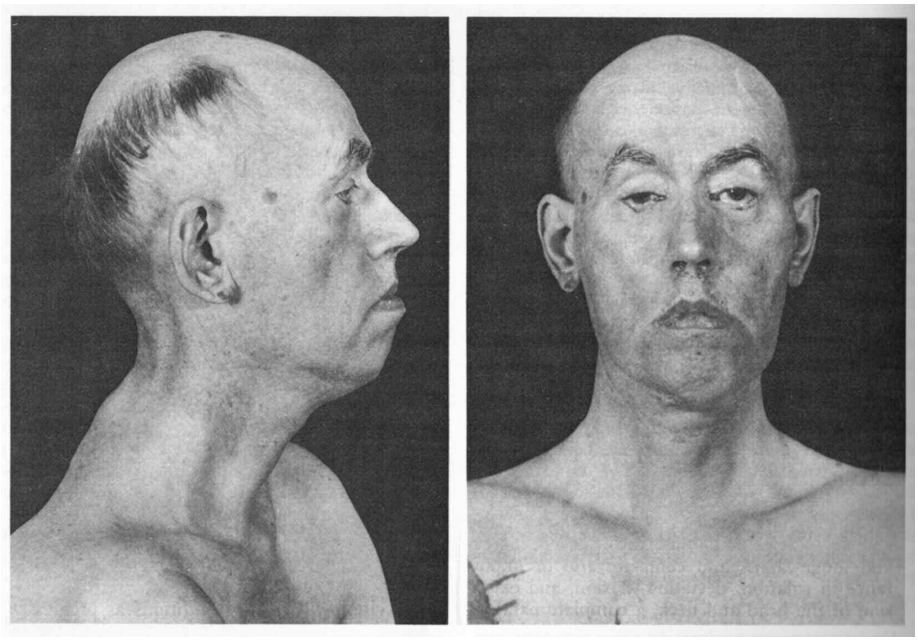


**Fig. 5.** Facies myotonica. (From: R.N. De Jong. Neurological examination, 1988).

**Fig. 6.** Woman, 45 y.o. (on the left), affected with myotonic dystrophy type 1 (adult onset), and her son, 17 y.o. (on the right), also affected, with a juvenile onset (anticipation phenomenon).

An over-expanded triplet (usually more than 1000 CTG) with early manifestation of symptoms at birth, with respiratory insufficiency and severe hypotonia (*floppy baby*) determines the condition of 'congenital myotonic dystrophy' (cDM1) which is documented only in DM1 (Fig. 7).

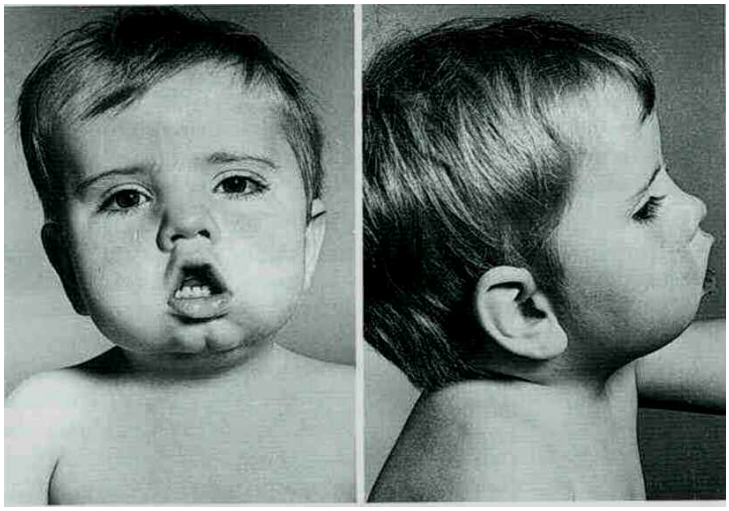


**Fig. 7.** A case of congenital myotonic dystrophy type 1 is shown.

In figure 8, a case of two siblings affected by DM1, both presenting a defective secretion of h-GH hormone, documented by GH-RH plus Arginine test, is shown.

**Fig. 8.** Two siblings affected by DM1. Both present a deficit in hypophyseal secretion of h-GH hormone, tested by GH-RH plus Arginine test.

Laboratory and instrumental clinical examinations, aimed at diagnosing a patient suspected for having DM1, involve:

1) routinary blood examination;
2) electromyography;
3) slit lamp study of the lens;
4) muscular biopsy (see section 'Pathology of DM' for details).

Routinary blood examination (1) generally reveals hyperCKemia (rarely superior than 1000 UI/L), suggestive for myopathic disturbances; a hypo-γ-globulinemia is not infrequently seen at seric electrophoresis.

Electromyography (2) documents a peculiar electrical spontaneous insertional activity within the relaxing muscle, explored by electrode-needle, generally diffused, but particularly evident in the small muscles of the hand and of the anterolateral region of the leg. Such activity is commonly named 'electromyografic myotonia' and appears as multiple myotonic discharges also known as '*dive bomber potentials*', characteristically variable for amplitude and frequency, within the single discharge (Fig. 9).

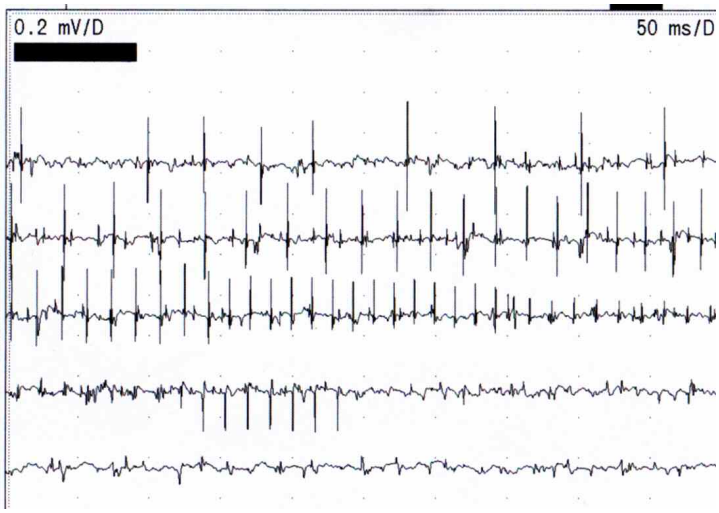



**Fig. 9.** Myotonic discharge.

The slit lamp study (3) permits to discover, in most of the cases, some posterior or subcapsular or central iridescent opacities of the lens, which is very typical for DM (Fig. 10). Historically, the first description of this phenomenon is due to Dr Fleischer (1918).

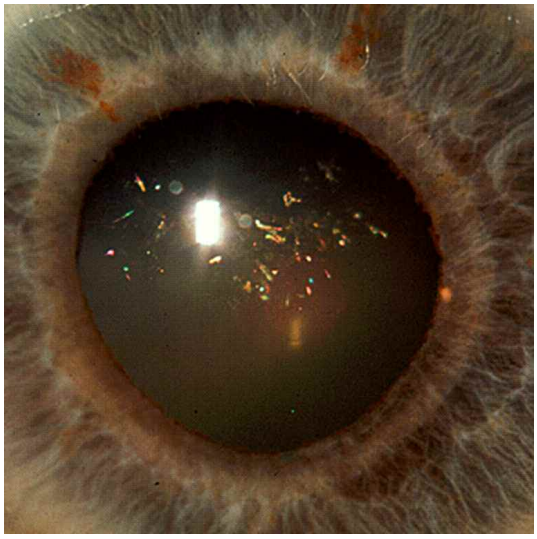


**Fig. 10**. *Slit lamp*: opacities of the lens in DM1.

It is supposed that a patient with an already documented DM undergoes a detailed clinical evaluation of cardiac, respiratory, neuropsychological and neuroperfusional functions, through:

1) cardiologic examination (ECG; ECG dynamic-Holter; echocardiography);
2) neuroimaging (MRI, SPECT);
3) neuropsychological examination.

A cardiologic study (1) is aimed at documenting the presence of occasional anomalies of conduction that especially occur in the atrio-ventricular tract (generally AV conduction blocks of first degree) or branch blocks (RBB, LBB) or more complexes defects of cardiac conduction (80% of patients affected by DM1); morphofunctional abnormalities (dilatative cardiomyopathy) are more rarely observed. Cases of sudden

death in young patients affected by DM1 are even described, while the cause of death in adults and elderly is generally due to pre-existent cardiac conduction defects (known or misdiagnosed). (Harper 2001)

Neuroimaging (2) and neuropsychological profile studies (3) of DM1 patients deserve special mention, concerning to the present work. Therefore, they will be treated separatedly beyond.

## *MYOTONIC DYSTROPHY TYPE 2 (DM2)*

To date, the epidemiology of Myotonic Dystrophy type 2 is not very well defined. Some Authors suppose that DM2 presents the same prevalence as DM1 (8-10 affected people per 100.000 inhabitants), but there might be some differences in territorial distribution for a possible founder-effect, thus determining higher prevalence in some regions (such as in Canada or in Germany) than elsewhere. However, the expanding capacities in doing correct diagnoses could even influence this data.

DM2, as well as DM1, is characterized by autosomal-dominant (AD) inheritance, but it seems that an anticipation phenomenon (earlier and severer involvement in offspring) can be excluded for DM2.

The onset of DM2 is quite variable. Differently from DM1, we cannot distinguish any kind of subgroups of patients on the basis of the onset. Usually, the symptoms onset occurs in adult life, more infrequently in younger people. Some patients receive their diagnosis on the base of only genetic analysis. In these cases, the clinical suspect is given by a determined familiarity for DM2.

DM2 is determined by the CCTG quadriplet expansion sited on chromosome 3q21, at the genomic locus of a zinc-finger-protein-9 named ZNF9. Alleles range in size from 75 to 11000 CCTG repeats (the largest known repeat expansion associated with human disease) (Day 2005).

The patients affected by DM2 cannot even be arbitrariously subclassified on the basis of CCTG quadriplet repeats, as it happens in case of CTG expansions in DM1. No ‘premutations’ ranges are taken into account for DM2. It is still debated either ‘congenital myotonic dystrophy type 2’ exists or not.

The phenotypic pleiotropic characteristics of DM2 are similar to those observed in DM1, and include:

1) proximal muscles hypotrophy, with distal progression and relative weakness (rarely of severe degree); occasional grip myotonia or percussion myotonia; calf hypertrophy is common, but not specific;
2) stiffness, cramps and myalgias, with or without fatigue, are frequently reported;
3) blepharoptosis and myopathic mouth (facial weakness) can be occasionally seen;
4) opacity of the lens or mature cataract;
5) hypogonadism, infertility, diabetes and other endocrine disturbances;
6) osteoscheletal abnormalities;
7) gastroenteric disturbances;
8) frontal balding, triangle-shaped face or hatchet face, slurred speech, rhinolaly are not usual distinctive features of DM2;
9) some data indicate the presence of cognitive abnormalities;
10) daily somnolence, hypersomnia are not reported.

Three cases of DM2 are shown in the following figures 11 and 12.

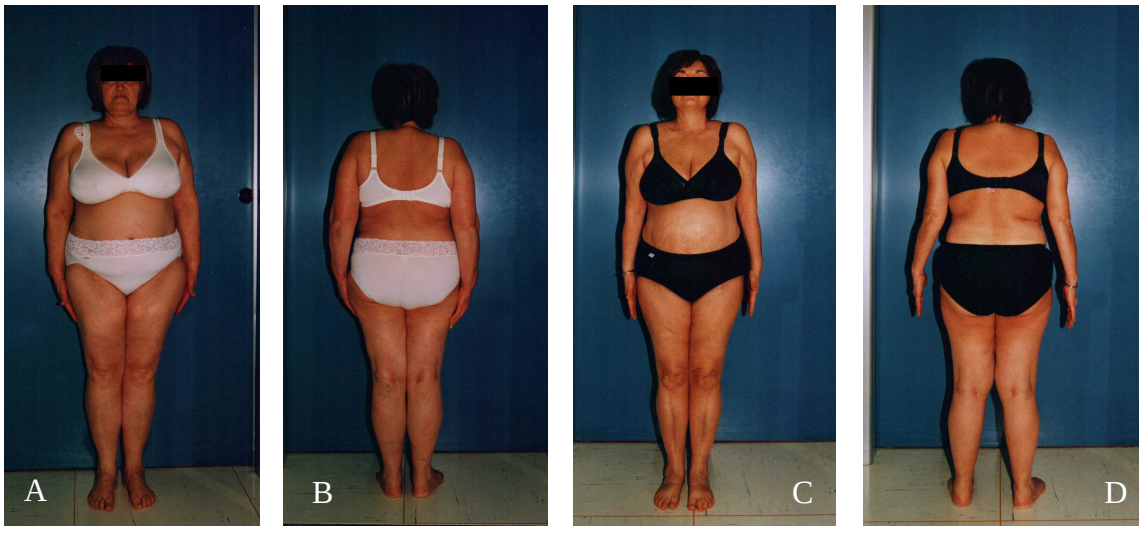



**Fig. 11**. Two sisters affected by DM2. A very mild hypotrophic quadriceps is shown in A and in C. Mild calf hypertrophy can be seen (B and D).

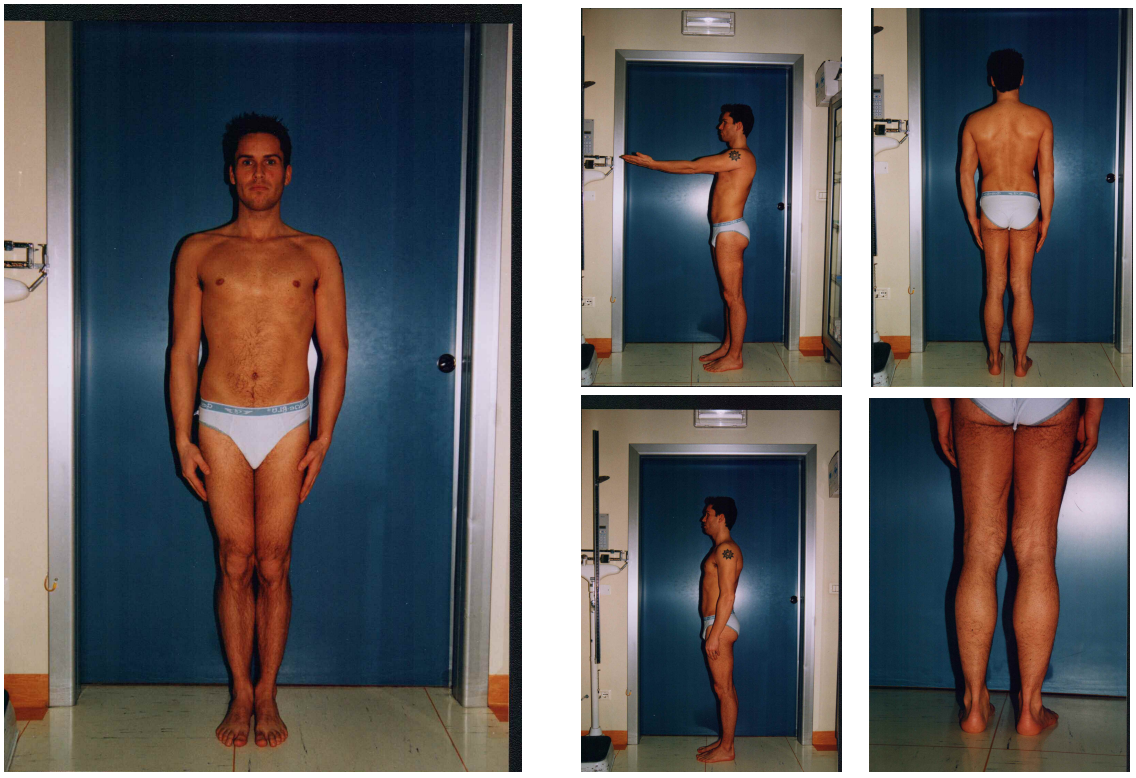


**Fig. 12**. O.A., 31 y.o., affected by DM2. The patients complains of occasional cramps and myalgias, but he does not have any physical limitation and practices sports. His clinical picture is almost normal. Muscular trophism and strength are globally well preserved. A very mild calf hypertrophy and a low grade scoliosis can be seen.

Laboratory and instrumental clinical examinations, aimed at diagnosing a patient suspected for having DM2, involve, as for DM1:

- routinary blood examination;
- electromyography;
- study of the lens through slit lamp;
- muscular biopsy (see section 'Pathology of DM' for details).

As in DM1, hyperCKemia, suggestive for myopathic disturbances, can be seen also in DM2; a hypo-γ-globulinemia is occasionally documented.

In DM2, myotonia can be fluctuating and intermittent and the patient can be symptom-free for very long periods. It is mandatory to perform an electromyography, which can detect myotonic discharges (*dive bomber potentials*) in about 50% of cases.

As in DM1, the slit lamp study is necessary to find any iridescent opacity of the lens, or cataracts.

In depth clinical evaluation of cardiac, respiratory, neuropsychological and neuroperfusional functions, are needed in DM2, as in DM1:

4) cardiologic examination (ECG; ECG dynamic-Holter; echocardiography);
5) neuroimaging (MRI, SPECT);
6) neuropsychological examination.

Table 2 shows a comparison of muscular and systemic involvement between DM1 and DM2.

**Tab. 2.** Comparison of muscular and systemic involvement between DM1 and DM2

| | DM1 | DM2 |
|---|---|---|
| Muscular weakness | | |
| mostly proximal at onset | - | + |
| mostly distal at onset | + | - |
| neck flexors | ++ | + |
| facial weakness | ++ | ± |
| muscles of the jaw | + | ± |
| extraocular muscles | - | - |
| ptosis | + | ± |
| Muscular atrophy | | |
| mostly proximal at onset | - | + |
| mostly distal at onset | ++ | - |
| sternocleidomastoid | ++ | ± |
| temporal muscles | ++ | ± |
| facial muscles | ++ | + |
| Hypertrophic muscles | | |
| calf hypertrophy | - | + |
| Myotonia | | |
| grip myotonia | ++ | + |
| orbicularis oculi | + | ± |
| tongue | ++ | + |
| massetere | + | + |
| muscles of the limbs | - | + |
| fluctuation | + | ++ |
| Cataract | + | + |
| Heart disturbances | ++ | ± |
| CNS | | |
| cognitive involvement | ++ | + |
| visuo-spatial deficit | ++ | + |
| hypersomnia | ++ | + |
| abnormal behaviour | ++ | + |
| mental retardation | + | - |
| Endocrine system | | |
| thyroid disturbances | + | ± |
| diabetes/insuline resistance | + | + |
| hypogonadism | + | + |

- = absent; ± = mild; + = present; ++ = markedly present (Meola G., Moxley R. J Neurol 2004)

## PATHOLOGY OF DM

### *DM1*

The association of some typical, although not pathognomonic, findings characterizes the histopathology of DM1, and permits to suspect a bioptical diagnosis of DM1.

- Centralized and/or internalized nuclei: many other myopathies can display such picture, but it seldom occurs as frequently as in DM1. Central nuclei are observed already at an early stage of the disease. Usually, the greater is the number of internalized nuclei, the greater is the muscular involvement of the patient. In a longitudinal section, it is possible to observe a typical chain-distribution, each of whom can contain until 20 nuclei. Actually, such phenomenon might not be exclusively due to nuclear division; a nuclear migration along the muscle fibre could be responsible of this pathologic finding. Moreover, the presence of a morphological heterogeneity of the nuclei has been reported: some of them are picnotic, some other appear pale and enlarged.
- Ring-fibres: these are fibres with myofibrils dislocated in shape of ring, firstly described by Heidenhain in 1918 and subsequently confirmed by Dubowitz and Brooke in 70% of the examined DM1 muscular biopsies, and correlated with chronicization of the disease.
- Fibre-polydimensionalism: there is a clear and early dishomogeneous distribution of fibre-diameter between type 1 and type 2 fibres, with typical low diameter of the first ones. Such discrepancy then proceeds in a marked atrophy of type 1 fibres, whereas a type 2 fibres hypertrophy can occur. This association seems to be very specific of DM1, since other muscular dystrophies or other myotonic disorders do not usually present similar pictures.

- Sarcoplasmic masses: these can coexist 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. Mussini *et al.* clarified the regenerating nature of the masses, by ultrastructural microscopy, in 1970.
- Beside the findings described above, several other myopathic phenomena, such as a 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).

*DM2*

Histopathological characterization of DM2 is controversial. Some abnormalisties in DM2 biopsies have been often reported as similar to the ones observed in DM1, but, a more specific pattern has emerged in the last years (Bassez 2008).

Predominant type 2 fibres hypotrophy has been described, in clear contrast with the type 1 fibre atrophy, typically seen in DM1.

Moreover, fibre polydimensionalism, centralized nuclei, small and occasionally angulated fibres, picnotic nuclei, have been documented independently from the sampled muscle, the symptoms and the progression of the disease.

Basicly, type 2 fibres present a bi-modal diametrical distribution: almost normal diameter fibres on one hand, markedly atrophic fibres with agglomerates of picnotic

nuclei (*nuclear clumps*) on the other hand. The presence of only mild hypotrophic fibres has also been reported.

The *nuclear clumps*, which can also be seen in denervated muscle, have been documented in DM2 muscles, without any other neurogenic changes and seem to be associated with type 2 fibres. This peculiar picture has been recently named 'simil-denervation' by Schoser *et al.* (2004). Its diagnostic predictive value is yet to be determined, since fibre-polydimensionalism, angulated fibres, centralized nuclei, type 2 fibre atrophy and nuclear clumps are aspecific findings, commonly seen in several other muscle disturbances.

However, Bassez *et al.* recently demonstrated that the coexistence of such abnormalities significantly improves the bioptical specificity for DM2. In particular, the presence of both type 2 fibre atrophy and centralized nuclei gives the byoptical analysis the greatest sensitivity (1.0) and very high specificity (0.92).

Again, many type 2 fibres have been found normotrophic, thus suggesting that nuclear centralization and atrophy reflect different pathogenic mechanisms or, at least, different stages of the same process. Besides, the nuclear clumps appeared only at an advanced stage of type 2 fibres atrophy.

The observation of nuclear centralization and selective atrophy of type 1 and type 2 fibres, respectively in DM1 and DM2, suggests that DM1 and DM2 are characterized by opposite pathologic patterns.

Figure 13 shows a muscle sample of DM1, while figures 14 and 15 show a muscle sample taken from a DM2 patient (see legends for details).

The fluorescent in situ hybridization (FISH method) permits to detect the presence of RNA abnormal accumulation as '*foci*' within the nuclei of patients affected

with DM1 or DM2. In the first case , CTG-triplets '*foci*' are evidenced, while in case of DM2, CCTG-triplets '*foci*' are found. (Figure 16).

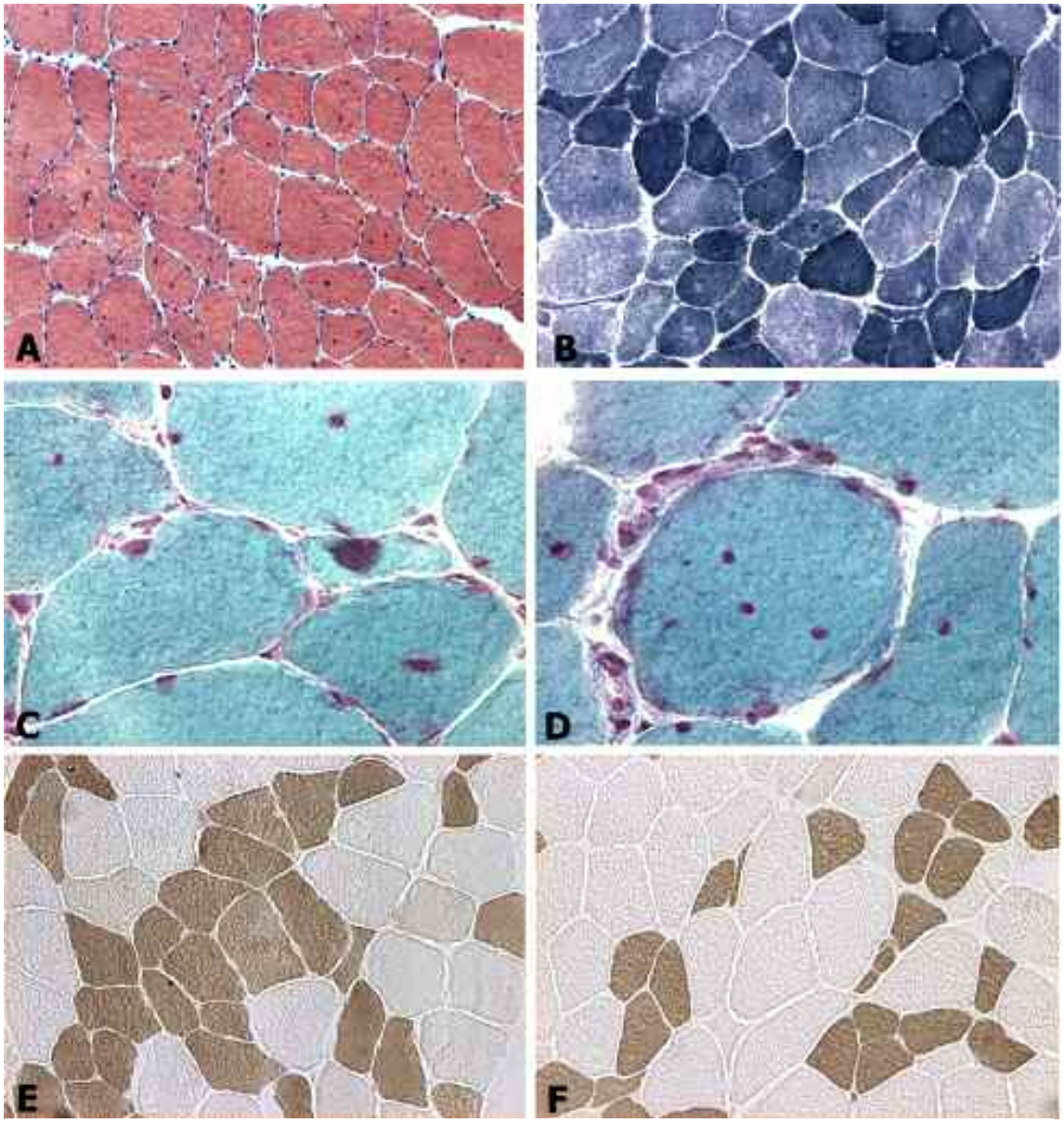



**Fig. 13.** DM1 muscle pathological findings. Transverse section of voluntary muscle fibres, seen in optical microscopy. **A.** Hematossilin-eosin (x10): the picture shows the presence of internalized nuclei, with fibre polydimensionalism. **B.** NADH-TR (x10): some scattered central nuclei can be seen, along with type 1 fibre hypotrophy and some moth-eaten fibres, scarcely reacting. **C.** Gomori trichrome (x40): sarcoplasmic masses, central nuclei, atrophic fibres are seen. **D.** Gomori trichrome (x40): the picture shows a single fibre with multiple internalized nuclei and sarcoplasmic masses, resembling a ring-fibre. **E.** Acid ATPase (x10): homotypic type 1 fibre-grouping. **F.** Acid ATPase (x10): type 1 fibre atrophy with type 2 fibre hypertrophy.

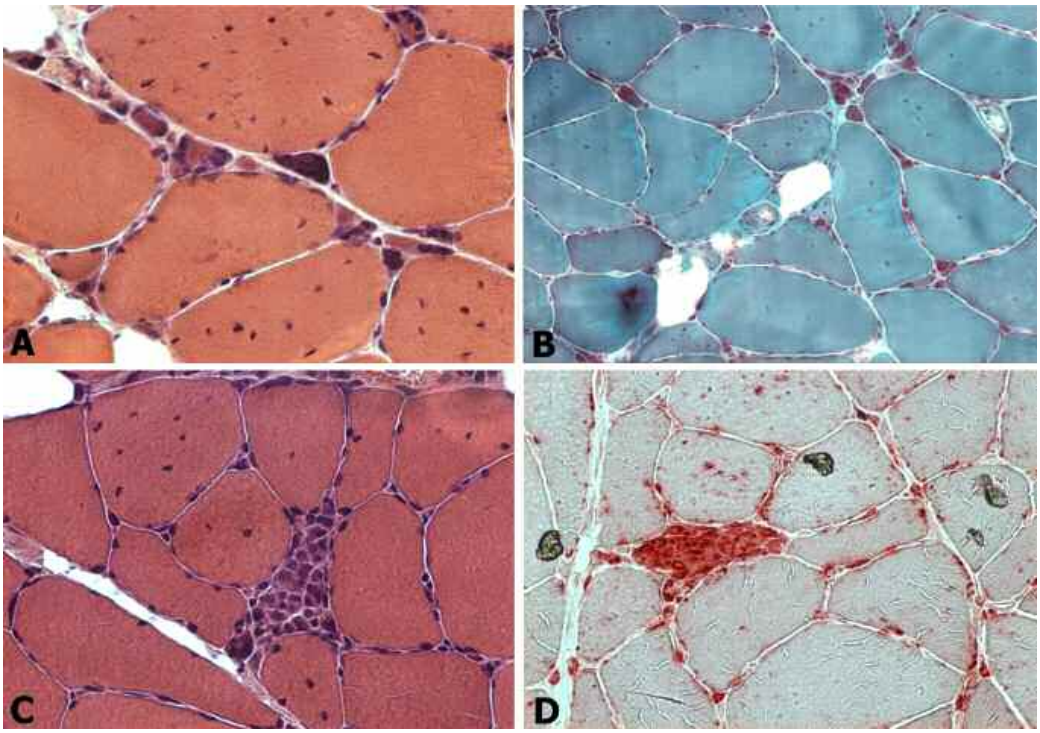



**Fig. 14.** DM2 muscle pathological findings. Transverse section of voluntary muscle fibres, seen in optical microscopy. **A.** Hematossilin-eosin (x40): this staining shows marked fibre polydimensionalism and the presence of multiple internalized nuclei and nuclear clumps. **B.** Gomori trichrome (x20): internalized nuclei, nuclear clumps and fibro-adipose changing can be observed. **C.** Hematossilin-eosin (x40): the picture shows a degenerating fibre in course of phagocytosis, invaded by macrophages. **D.** Acid phosphatase (x20): degenerating fibre during phagocytosis, with clear lysosomal activation.

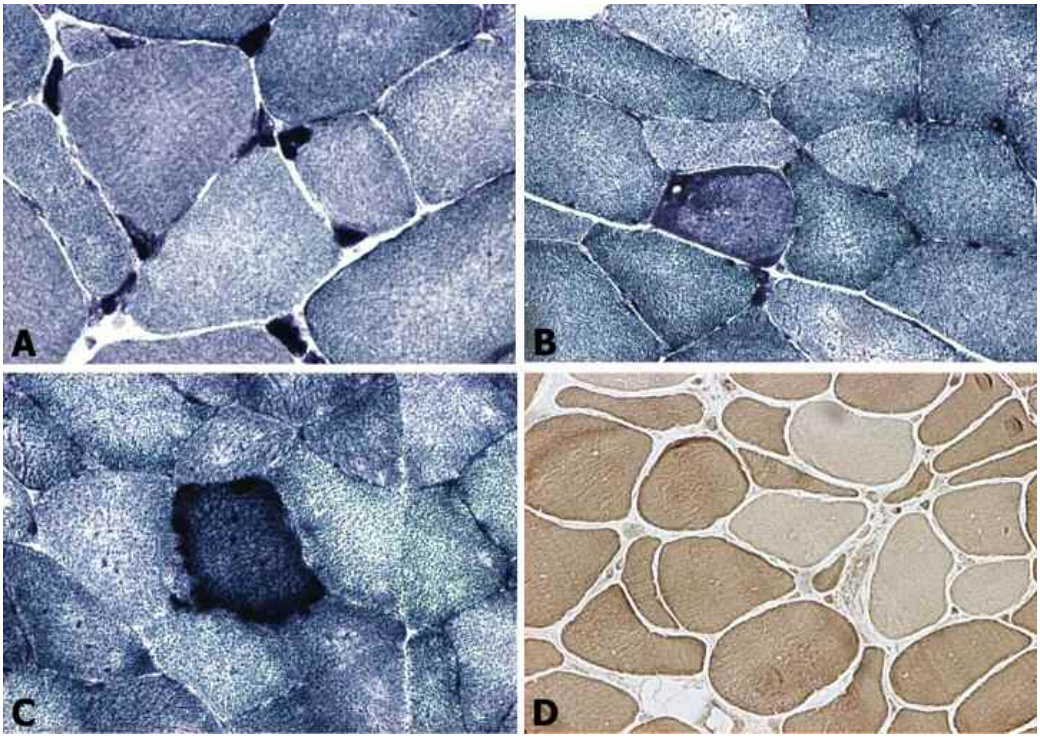



**Fig. 15.** DM2 transverse section of DM2 muscle. Optical microscopy. **A.** NADH-TR (x40): many nuclear clumps are evidenced. **B.** NADH-TR (x20): a ring-fibre **C.** SDH (x40): a 'ragged-red' fibre is shown. **D.** Basic ATPase (x20): type 2 hypotrophic fibres, with homotypic grouping.

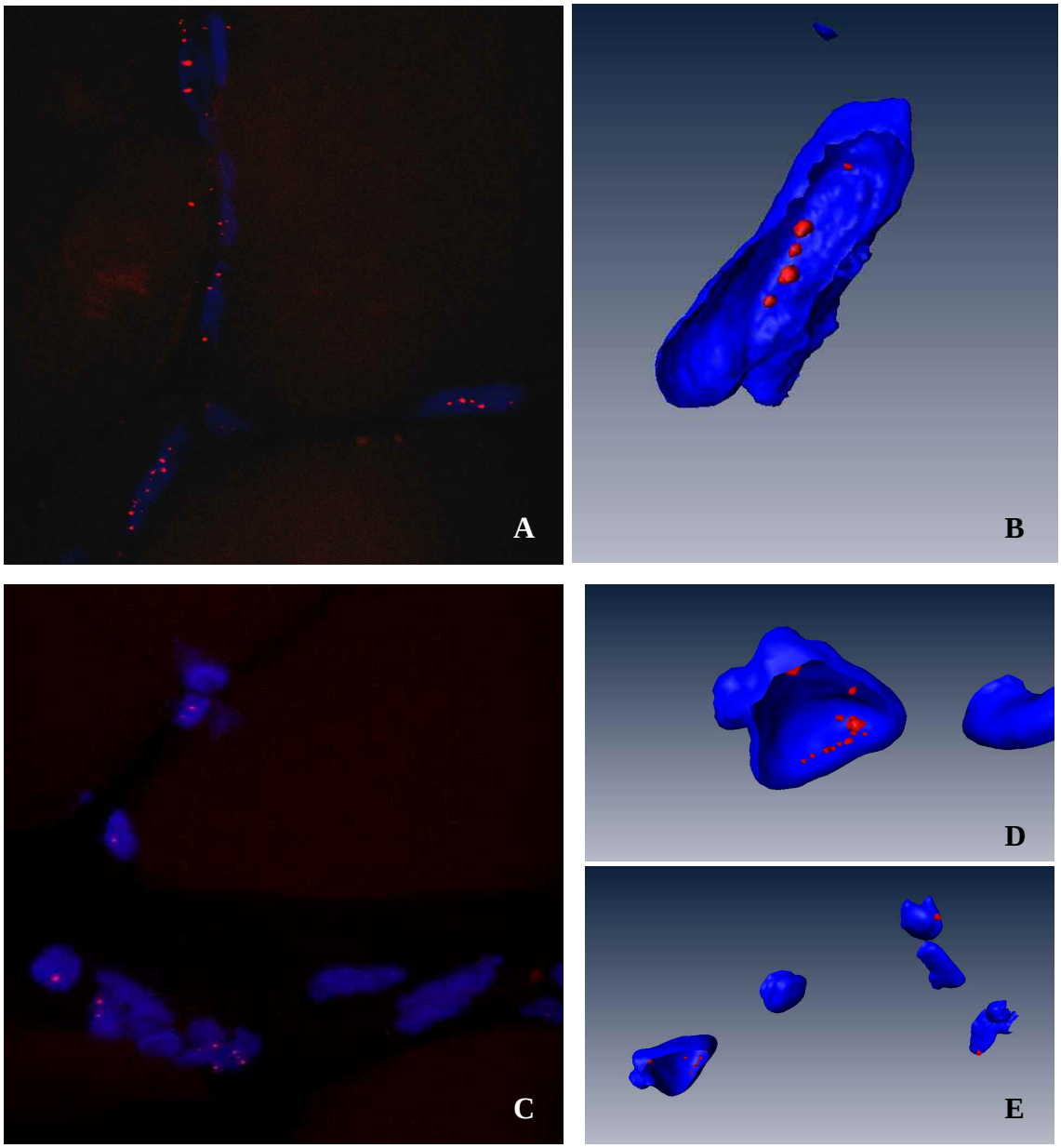



**Fig. 16.** *Foci* of pathologic accumulation of aberrant RNA (CTG-triplets) are evidenced by FISH method in a case of DM1 (panel A). In panel B, a 3D-reconstruction of the detected *foci*, to better describe number, shape and intranuclear localization of the *foci*. In panel C, *foci* of CCTG-quadriplet repeats are shown and evidenced by FISH. Panels D and E are 3D reconstruction, in the same case.

## PATHOGENESIS OF DM

Myotonic dystrophies (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 (Day 2005).

The pathogenesis of DM1 (and, actually, of all myotonic dystrophies) has not yet been completely understood to date, especially because of its particular complexity; for at least 10 years it has represented a particularly interesting aspect for molecular biologists, most of all for those who deal with hereditary disorders associated with genomic polynucleotidic expansions. The research done in the last 5 years has been extremely fecund and its several scientific discoveries permitted to shed some light about the pathogenic mechanisms that underlie the development of the syndromic manifestations of DM and, perhaps, of other genetic diseases due to triplet expansions.

The molecular biologists that study this matter generally agree in defining, as main pathogenic *focus*, the deposition of an abnormal transcribed but non-translated RNA from the sequence [CTG]n sited at DMPK-gene *locus*, within the nuclei of affected cells that express such gene.

The documentable epiphenomenon of this process would be characterized by the presence of intranuclear '*foci*' of RNA, pathologically deposited inside the nuclei of affected cells. (Figures 16 and 17).

The description of intranuclear '*foci*' of *CTG-repeats* transcripts goes back at least at 1995 and temporarily passed through silence, maybe unable of suscitating the deserved interest that it has more recently gained.

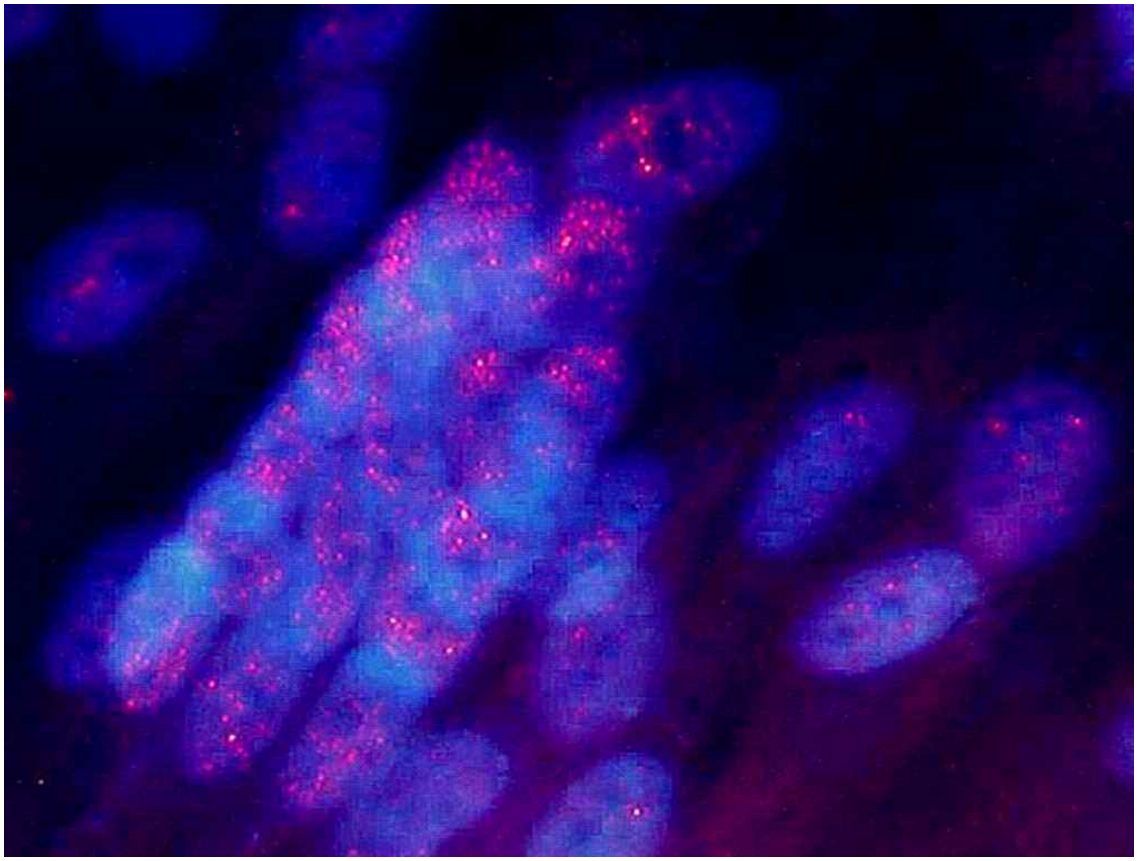


**Fig. 17.** FISH (Fluorescent In Situ Hybridization): *in situ* hybridization obtained on myoblasts of DM1 with 3000 *CTG repeats* by *Cy3-labeled peptide nucleic acid (CAG)5 probe* and staining by DAPI (Langlois et al.).

The idea that RNA can acquire, through an aberrant deposition process, an effetct of '*toxic gain of function*' is particularly fascinating because it would let to justify, in a simple but elegant way, the mutual complexity and similarity of poly-syndromic features of both DM, although the genes recognized as responsible of the two diseases are so far one from each other on the gene map and so functionally different the proteins which these two genes encode for.

The typical AD-inheritance mode supports this hypothetical mechanism: therefore, one single chromosome would be enough to determine the toxic effect due to the deposition of RNA excess.

At least two different pathogenic mechanisms have been historically hypothesized to explain the pathogenesis of DM1: 1) Aploinsufficiency of DMPK gene; 2) Aploinsufficiency of neighboring genes.

Hypothesis 1) postulates that the transcriptional defect of DMPK-gene is the alteration enough to determine at least part of the symptoms, in relation to the reduction of the DMPK transcripts and, consequently, of the protein: decreased rates of DMPK-mRNA in the myofibres of the patients, as well as the development of an arrhythmogenic cardiopathy in the DMPK-gene *knock-out* mouse model, seem to support this hypothesis. However, not only the *knock-out* mouse does not show the entire syndromic picture with all its complexity, but, moreover, the presence of a single base mutation in the DMPK-gene that could induce a phenotypic picture exactly corresponding to that of DM1, has never been documented in man. Hence, on the basis of such considerations, the hypothesis 1) has progressively lost credibility in time, especially with the more and more increasing knowledge in this molecular biology field.

The hypothesis 2) (*neighboring genes*) appears particularly suggestive, because of the possibility to more consistently justify the heterogeneity of the clinical features of DM1: the neighboring genes of DMPK are here considered (DMPK would act, in this case, only as '*primum movens*'). All the remaining symptoms not tributable to the DMPK defect at once, would be thus tributed to these neighboring genes, given besides the results on murine models. The presence of a SIX5-gene, similar both to the *sine oculis*-gene of the *drosophila melanogaster* and to a gene-family that regulates the development of the distal muscle fibres in mice, leads to the hypothesis that an abnormal transcription of this gene, in man, would be responsible for both the ocular component (cataract) and the muscular component (hypotrophy, weakness) in DM1.

Parallely, the DMWD-gene as well, DMPK neighboring itself, being expressed in the testes, could justify the reduction of fertility which is typically seen in patients affected by DM1. Finally, the FCGRT-gene, a gene that transcribes an Ig receptor, has been considered as a possible cause of hypo-γ-globulinemia observed in DM1. However, although interesting, the speculations about the transcriptional defects of the DMPK neighboring genes as potential co-factors in the DM1 pathogenesis induction, appeared quite non-convincing, for some contradictory laboratory results.

At present, the third and most believed pathogenic hypothesis (*toxic gain of function of CUG-repeats*) tries to find an agreement between the early observations about the intranuclear accumulation of RNA as '*foci*' and the subsequent acquisition of molecular biology. Infact, parallely to the study of both behaviour and metabolism of endonuclear RNA, several researches aimed at defining the role of some endonuclear proteins targetting RNA and probably involved in endonuclear transfer mechanisms (*trafficking*), post-transcriptional modifications, newly-synthetized RNA molecules catabolism, were developing. Among these, the dsRNA-BPs (*RNA binding proteins*, like PKR, TAR, RNA helicase A) and, most of all, proteins of the MBNL family (*muscleblind proteins*, particularly MBNL1) suscitated special interest. The observation that intranuclear '*foci*' of aberrant RNA within the myonuclei, evidenced by FISH method, are able to co-localize with aggregates of MBNL evidenced by immunofluorescent specific antibody, rather than different protein families (such as *CELF proteins*, which bind RNA UG-rich, and *dsRNA-BPs*, which electively bind *double-stranded RNA*) suscitated great enthusiasm. Conversely, it was documented on one hand an increased intranuclear concentration of dsRNA-BPs, on the other hand, a decreased intranuclear concentration of MBNL. The Authors that dealed with this

problem suggest that, at present, the only reasonable hypothesis is that a ‘sequestration of MBNL’ by aberrant RNA produced by the CTG triplet expansion occurs. In this case, the lager is the CTG triplet expansion on chromosome 19q, the stronger is the efficacy of the sequestration. Therefore, in this way, the MBNL normal activity on the healthy RNA would be torn apart with a ‘subtractive’ mechanism. In this balance, dsRNA-BPs, recalled into the nucleus, would result definitely increased, with a subsequent ‘loss of stechiometric balance’ of the several parts and a compromission of the processing of many other neotranscribed endocnuclear RNAs.

This interesting model can be represented as follows in figure 18.

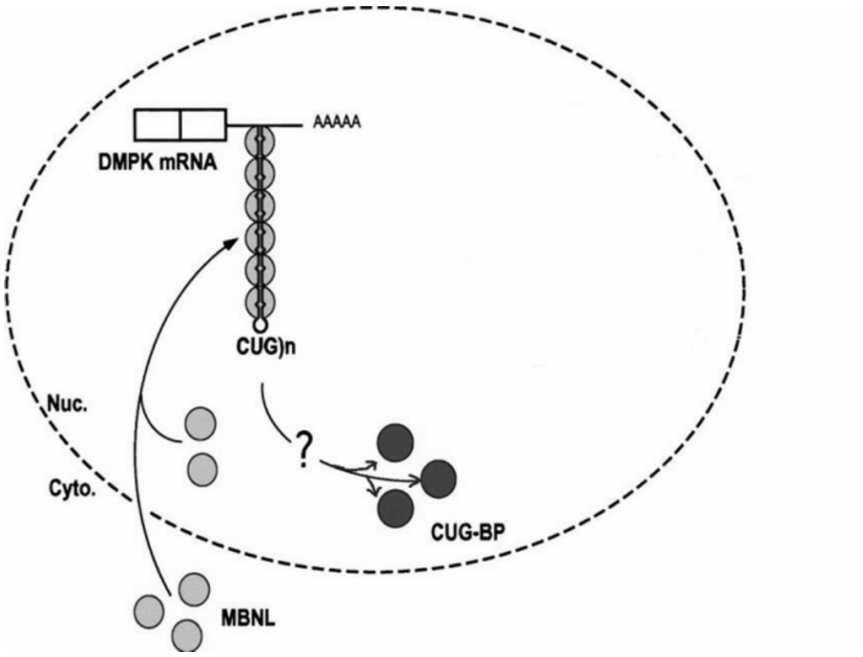



**Fig. 18**. Pathogenic model of DM1: MBNL sequestration by dsRNA and subsequent intranuclear reduction of concentration; secondary increase of free CUG-BP nuclear concentration.

Besides, in the last few years, many studies have been aimed at understanding the very fascinating problem of the alternative splicing dysregulation mechanisms that seem to be involved in the pathogenesis of both myotonic dystrophies. To date, the results of these studies seem to quite clearly correspond to the clinical profiles and to the syndromic complexity that the DM patients show.

In particular, it was very interesting to discover that some primary mRNA transcripts, normally present in cells (or myoblasts) of healthy controls, and subjected to post-transcriptional modifications for alternative splicing able to produce proteic isoforms specific for that cell-line, in case of myoblasts of DM1 patients they were subjected to an abnormal post-transcriptional process, so that some aberrant secondary transcipts were made, unable to determine the original proteic isoform. The new proteic isoform, sometimes atypical for that cytotype, sometimes paradoxically original, would have lower chances to result in a functional protein in the cellular context where it has been accidentally produced or -even worse- it could be completely inefficient.

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

A specific symptom, among the ones previously described in the paragraph dedicated to clinical aspects of DM, would correspond to each of the specific damages secondary to abnormal ribonuclear processes.

In detail: 1) the anomaly of the proteic isoform of the chloride channel (ClC-1) would be responsible of the myotonic phenomenon, secondary to a modification of the opening-closing kinetics of the voltage-dependent channel; 2) the alteration of the insulin receptor would determine the insulin-resistance, for difficulty of the receptor

itself (IR) in conformationally interacting with its physiological ligand (insulin); 3) the anomaly of cardiac T-troponin (cTNT) could be implicated in the development of the arrhythmogenic cardiopathy typical of DM1; 4) abnormalities of tau-protein associated to microtubules would justify the cognitive defects present in DM1, which will be better debated forward; 5) finally, the myotubularin (*myotubularin-related-1*, MTMR1) dysregulation mechanisms would be responsible of the severe congenital phenotype and of its marked muscular atrophy and weakness which represent dramatic features of cDM1.

It has to be specified that the last three supposed molecular mechanisms are yet to be demonstrated.

The processes that determine the appearance of opacities of the lens, gonadal insufficiency and hypo-γ-globulinemia, are still rather mysterious. Studies aimed at resolving such aenigma are still ongoing.

The results related to the intranuclear '*foci*' of aberrant RNA on one hand, and the results related to the abnormal processing of primary transcripts of mRNA on the other hand, seem to be sequentially linked at molecular level by a simple cause-effect relationship: the stechiometric dysequilibrium among the several RNA-binding proteins, which lead RNA metabolic destiny within and outside the nucleus, would disclose as an abnormal interaction among the various macromolecular complexes designated to spliceosomes formation, with the subsequent development of several atypical mature mRNAs, and of their related proteic non-functioning isoforms. (Figure 19).

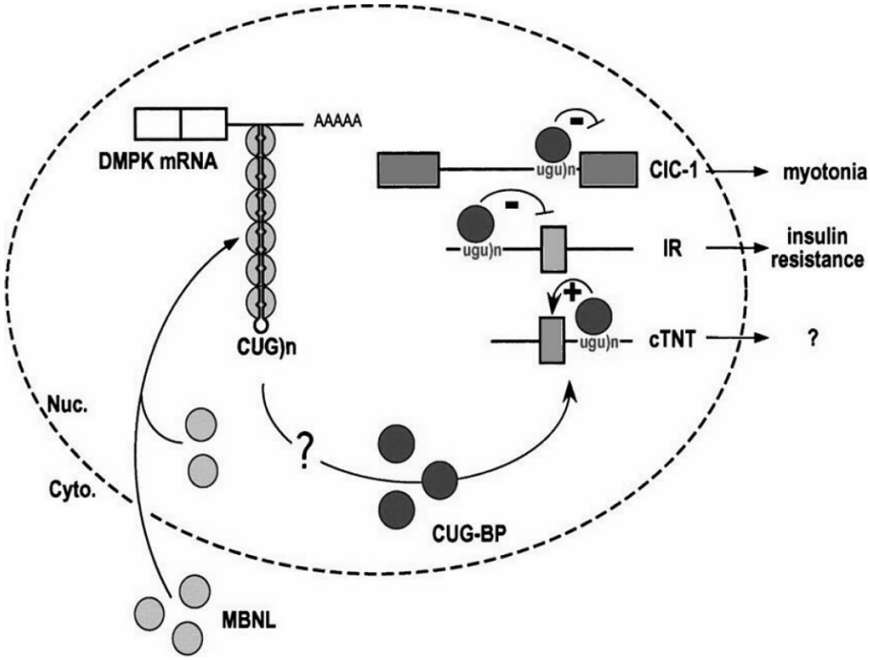



**Fig. 19**. Pathogenic model of DM1: MBNL sequestration and increase of CUG-BP are followed by the alteration of primary mRNA transcripts processing, with subsequent development of mature but pathologic endonuclear mRNAs, from whom several atypical proteic isoforms can derive.

The DM researchers thought that the deposition of aberrant RNA endonuclear '*foci*', the sequestration of MBNL and the consequent 'compensation' increase of other RNA binding proteins, and therefore the secondary dysregulations of RNA processing that conducts to the formation of unusual and malfunctioning proteic isoforms, should be extended from DM1 to other different forms of DM (DM2/PROMM/PDM…) also associated to polynucleotidic CTG triplet or CCTG quadriplet repeats.

Hence, the two principal clinical forms of DM, although originating in a completely different gene locus (chromosomes 19 and 3), would determine complex and surprisingly similar syndromic features, since a 'common final pathway' can be recognized in their pathogenesis, as it is well explained by Day *et al.* in the following figure (Fig. 20).

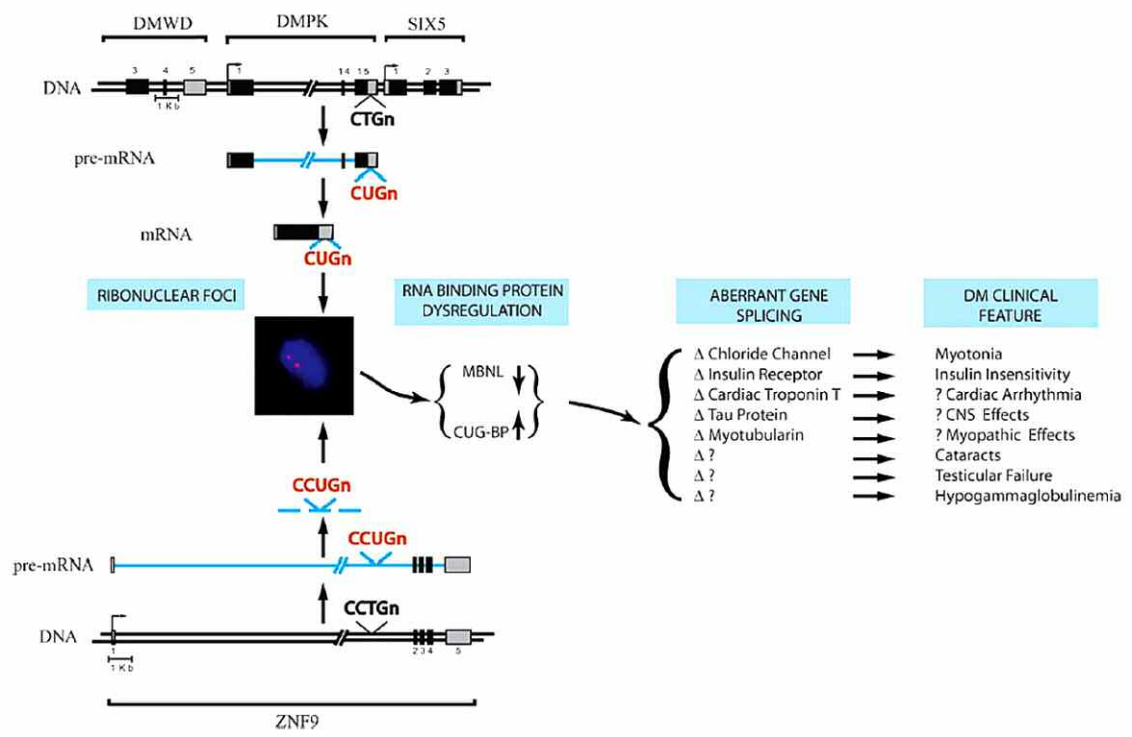



**Fig. 20**. Pathogenesis of DM: two distinct *loci* for a common final pathway.

# BRAIN IN DM

## *COGNITIVE DISTURBANCES IN DM*

The attempt of classifying the patients affected by DM1 through psychiatric methods goes back at least to 1937, when mental disturbances within DM1 families were initially described. (Maas 1937).

Successive descriptions were found in 1966 and then in 1982 by Woodward, who was interested in the matter. Several studies aimed at defining cognitive profiles and personality traits in DM1 were produced in the eighties.

In 1992 Colombo *et al.* studied 40 patients, comparing them with 20 healthy controls, subjecting them to neuropsychological and psychiatric batteries. They concluded that the intellectual impairment and the psychiatric disturbances found in DM1 represent a particularly important aspect in severe forms of DM1.

In 1994 Turnpenny *et al.* compared 55 DM1 with 31 healthy controls and found significant differences by using the Wechsler Adult Intelligence Scale Revised (WAIS-R) and arithmetical performance test; he detected a negative correlation between the calculated I.Q. and the CTG triplet expansion and a positive correlation with age of onset of symptoms. Besides, this Author documented that the CTG triplet expansion is a bad predictor of I.Q.

In 1998 Delaporte *et al.* researched a specific personality pattern in DM1 in 15 patients by the International Personality Disorder Examination and for the first time suggested that these patients are affected by an avoidant personality disorder (which is not commonly found in people), with obsessive-compulsive, passive-aggressive and schizotypic traits, which could not be justified by concurrent neuromuscular

impairment. Perini *et al.* partially confirmed in 1999 what had been previously observed.

Concerning to DM2, a very few studies have been produced to dissect the question whether a specific neuropsychiatric personality trait characterizes DM2 as well as DM1 patients. Meola *et al.* reported some defects in visuo-spatial abilities, similar to those observed in DM1, probably secondary to mild brain perfusion changes (scattered areas of hypoperfusion in the frontal and posterior planes) documented by PET and SPECT studies. (Meola 1999 and 2003).

Table 3 summarizes methods and results of the principal studies conducted in the past years by many Authors, aimed at studying the brain involvement of patients affected by DM1 or DM2, by both instrumental and functional techniques.

### *NEUROIMAGING IN DM*

In this section, special mention is deserved to *neuroimaging* in DM. From the eighties, many studies were done to dissect this problem. They concern both morphological (MRI), perfusion (SPECT) and metabolic aspects (PET) of CNS of DM patients. In DM, a correlation between neuroradiological findings and neuropsichological-cognitive profiles was also researched, but an association between degree of cortical atrophy (MRI) and severity of intellectual impairment, which has rather to be better related to other morphological anomalies (thickening of the skull, focal lesions of the white matter, commonly seen in the temporal poles), was not demonstrated. Perfusion studies mostly show cortical hypoperfusion in the frontal and associative temporo-parietal left regions, with major brain damage in case of congenital forms, in DM1; minor degrees of brain damage in DM2.

In particular, brain involvement in DM has been studied by MRI (Magnetic Resonance Imaging), SPECT (Single Photon Emission Computed Tomography) and PET (Positron Emission Tomography). Since 1988 the presence of white matter hyperintense lesions (WMHLs) and the presence of cortical atrophy and dilatation of ventricular spaces have been detected in DM1 patients by brain MRI (Glantz 1988). Recurrence, localization, diffusion, morphology and relationship with other features of the disease, appear quite controversial in several studies (Glantz 1988, Huber 1989, Sinforiani 1991, Fiorelli 1992, Chang 1993, Damian 1994, Damian 1994, Abe 1994, Censori 1994, Hashimoto 1995, Bachmann 1996, Hund 1997, Miaux 1997, Chang 1998, Ogata 1998, Meola 1999, Martinello 1999, Di Costanzo 2001, Di Costanzo 2002, Kassubek 2003, Kornblum 2004, Antonini 2004, Kuo 2005, Fukuda 2005, Vielhaber 2006, Ota 2006, Giorgio 2006, Di Costanzo 2008, Kuo 2008). Huber *et al.* described 'unusual' WMHLs in anterior or medial portions of temporal lobes (Huber 1989). Subsequently, many other Authors detected and studied the ATWMHLs (Anterior Temporal White Matter Hyperintense Lesions) (Glantz 1988, Censori 1994, Miaux 1997, Ogata 1998, Di Costanzo 2001, Di Costanzo 2002, Kornblum 2004, Kuo 2005, Di Costanzo 2008, Kuo 2008). Despite the large number of reports, the relationship between the severity of WMHLs and the onset of disease is not clear. Cortical atrophy in DM1 is a common finding, 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 (Huber 1989, Sinforiani 1991, Damian 1994, Censori 1994, Bachmann 1996, Miaux 1997, Meola 1999, Martinello 1999, Di Costanzo 2002, Kassubek 2003, Kornblum 2004, Antonini 2004, Di Costanzo 2008, Kuo 2008). Since 1997, the presence of focal WMHLs has been documented by brain

MRI, in both DM2 and DM1 (Hund 1993). Further imaging studies have shown that the degree of severity, morphology and extent of WMHLs are variable, but it is generally agreed that the pattern of brain involvement recorded by MRI (atrophy and focal WMHLs) is milder in DM2 than in DM1 (Meola 1999, Kassubek 2003, Meola 2003, Kornblum 2004, Vielhaber 2006). Only one SPECT study has demonstrated significantly low cerebral blood flow (CBF) in DM1, compared to normal controls (Chang 1993). In the DM1 patients studied, 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 (mDM1) (Chang 1993). Three PET studies have reported a reduction in the cortical glucose utilization rate (CMRGlu) in DM1 (Fiorelli 1992, Mielke 1993, Annane 1998), in a CTG-dependent manner (Annane 1998). A similar pattern of hypoperfusion with a reduction in regional cerebral flow in the temporal poles and orbito-frontal and medial frontal regions has been shown by PET perfusion studies in DM1 and DM2 patients, showing a relatively selective alteration in visual-spatial functions. In these patients, the cognitive changes did not correlate with structural abnormalities on brain MRI (Meola 1999). These findings were later confirmed in DM2 by SPECT scans, with frontal rCBF reduction (Meola 2003). Table 3 provides a summary of the neuroimaging studies conducted on DMs.

The case of a patient affected by DM and studied by brain SPECT is reported in figure 21. Figure 22 shows a brain PET scan in a DM1 patient.

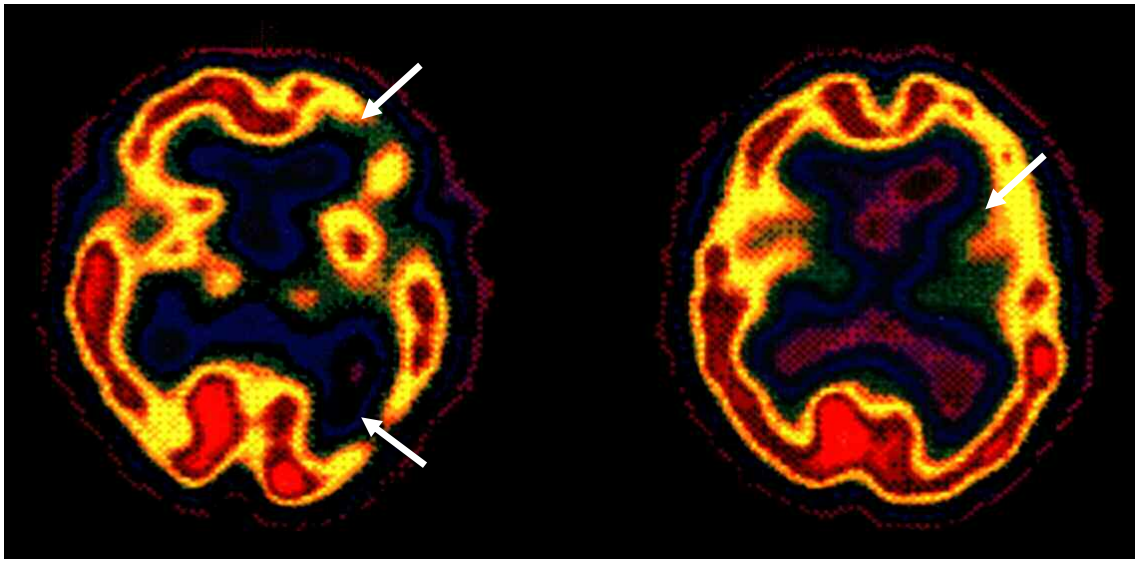


**Fig. 21**: Brain perfusion $^{99m}$Tc –SPECT of a patient affected with DM1: frontal and parieto-temporo-occipital (global left) hypoperfusion is shown (arrows).

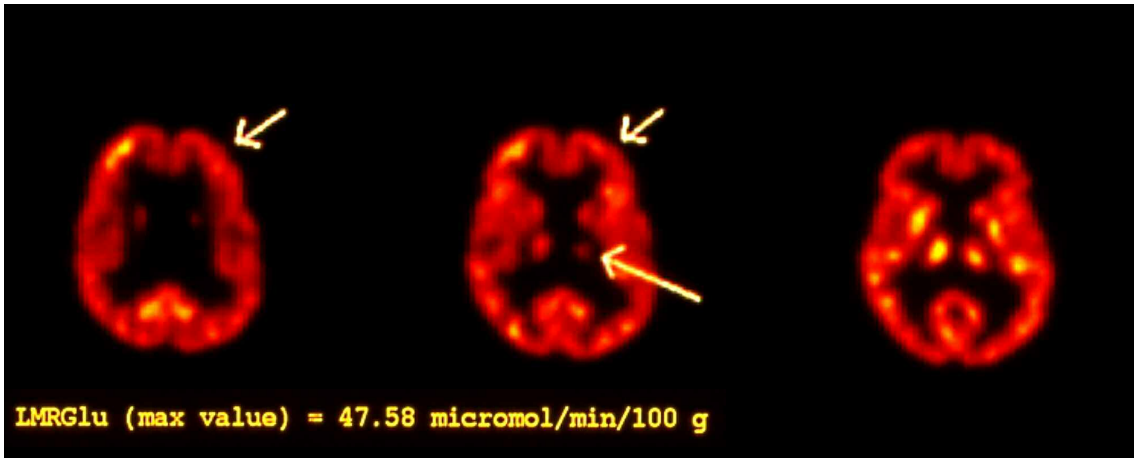



**Fig. 22**: Brain $^{18}$FDG-PET scan of a patient affected with DM1: left frontal and thalamic hypometabolism is shown (arrows).

Table 3. Summary of previous neuroimaging studies results in DMs.

| Author | Year | Reference | Population | Methods | Results |
|---|---|---|---|---|---|
| Glantz *et al.* | 1988 | Arch Neurol | 14 DM1, am-cntrls | MRI | Increased evidence of ventriculomegaly and lumpy and/or thick pattern of periventricular hyperintensity |
| Huber *et al.* | 1989 | Arch Neurol | 44 DM1 | MRI, Neuropsy | 10 pts with intellectual impairment but no correlation with neuromuscular status. MRI degree of cortical atrophy not correlated to cognitive involvement. WMHLs, skull thickness and anterior temporal lobe abnormalities significantly related with intellectual status. |
| Sinforiani *et al.* | 1991 | Funct Neurol | 37 DM1 | 12 MRI, 37 Neuropsy | 7/12 pts with WMHLs. Cognitive performances at low average level (particularly in DM1 with brain atrophy) |
| Fiorelli *et al.* | 1992 | Neurology | 11 DM1, 14 cntrls | FDG-PET, MRI | Cortical glucose utilization rate reduced by about 20% (PET). Mild cortical atrophy with no correlation to CMRGlu (MRI) |
| Mielke *et al.* | 1993 | Psych Res Neuroim | 3 DM1 | FDG-PET | Impairment of rCMRGlu in all cortical and subcortical regions (particularly in frontal cortex and lentiform nucleus) |
| Chang *et al.* | 1993 | Arch Neurol | 22 DM1 (14 pDM1, 8 mDM1) | 22 SPECT, 17 MRI, 19 Neuropsy, 10 am-cntrls | Lower neuropsychological performances in DM1 than in controls. Reduced CBF in DM1 than controls (in mDM1>pDM1). Most severe changes of CBF in frontal and temporo-parietal association regions. (strong correlation with I.Q.). |
| Damian *et al.* | 1994 | Neuroreport | 28 DM1 | MRI, Neuropsy | 14 with WMHLs; 14 with atrophy. Neuropsychological results correlated to brain damage and to CTG size when CTG >1000. MRI with poor correlation with genetics. Importance of disease duration. |
| Damian *et al.* | 1994 | Acta Neurol Scand | 22 DM1, 39 MS | MRI, Neuropsy | 73% of DM1 with WMHLs: correlation of lesion extent with cognitive deficits ('subcortical dementia-type' in severest cases). Subcortical WMHLs and total extent of lesions have better correlation with cognitive dysfunction than cerebral atrophy and periventricular lesions. Iportance of pattern extent of WMHLs. |
| Abe *et al.* | 1994 | J Neurol Sci | 14 DM1 | MRI, Neuropsy | All DM1 with ventricular enlargement and WM abnormalities. Worse cognitive performances correlated only with major degrees of WM changes. |
| Censori *et al.* | 1994 | Acta Neurol Scand | 25 DM1, 25 cntrls | MRI, Neuropsy | 84% DM1 with WMHLs, involving all cerebral lobes (no side preference). 28% with ATWMHLs. Presence of cortical atrophy (no relationship with WMHLs). No correlation found between clinical neurological profile and extent of brain abnormalities (WMHLs/atrophy). Temporal poles involvement does not characterize any specific neuromuscular/cognitive profile, does not correlate with onset age and disease duration. |
| Hashimoto *et al.* | 1995 | Brain & Develop | 13 DM1 (7 CDM1, 6 ADM1) | MRI, Neuropsy | 7/7 CDM1 with ventriculomegaly (CDM>ADM1); 7/7 CDM1 with low I.Q. (CDM1 lower than ADM1); 6/7 CDM1 with WMHLs; 4/7 CDM1 with small corpus callosum; (CDM1>ADM1); 2/7 CDM1 small brainstem; 1/7 CDM1 cerebellar WMHLs; 5/6 ADM1 with WMHLs (one with ventriculomegaly). |
| Hashimoto *et al.* | 1995 | Brain & Develop | 6 female DM1, 10 asm-cntrls | MRI | Decreased volume width of cerebrum, corpus callosum, pons, pituitary gland respect to controls. |
| Bachmann *et al.* | 1996 | Neuroradiol | 40 DM1 | brain MRI, muscle MRI, Neuropsy | 68% with diffuse brain atrophy (correlated with mental retardation, disease duration and CTG expansion size); 38% with wide Virchow-Robin spaces; 65% with WMHLs; 35% with skull thickening. |
| Hund *et al.* | 1997 | Neurology | 3 DM2 families | MRI | 4 DM2 with marked white matter T2 weighted images hyperintensity; 2 DM2 similar but mild to moderate changes; several clinical symptoms. |
| Miaux *et al.* | 1997 | Neuroradiol | 13 ADM1, 13 asm-cntrls | MRI, Neuropsy | Cerebral atrophy. Thickening of the skull. Ossification of the falx cerebri (2 pts). 70% signal of WMHLs kind. 5/13 WMHLs in temporal poles. Relationship with cognitive status in 1 pt only. |
| Annane *et al.* | 1998 | Neuromusc Dis | 11 DM1, 11 cntrls | FDG-PET | CMRGlu is reduced in DM1 with CTG dependent manner. |
| Chang *et al.* | 1998 | Arch Neurol | 14 DM1, 24 cntrls | Spectroscopic MRI | Elevated levels of myoinositol, of total creatine, of choline containing compounds in DM1. Creatine and myoinositol peak areas correlated with CTG size especially in temporo-parietal regions. |
| Ogata *et al.* | 1998 | Neuroradiol | 12 DM1 | MRI, MMSE | 8/12 mild intellectual impairment; 10/12 presence of WMHLs; 7/12 ATWMHLs with MMSE 22-26; 4 pts with no cognitive impairment and no ATWMHLs. |

## *NEUROPATHOLOGY OF DM*

Ono *et al.* reported cell loss in specific areas of the brain at *postmortem* of patients with DM1, such as in the dorsal raphe nucleus, superior central nucleus, dorsal and ventral medullary nuclei, and subtrigeminal medullary nucleus. Other Authors reported neuronal loss in the superficial layer of the frontal, parietal and occipital cortex as well as in the substantia nigra and locus coeruleus. Neuronal eosinophilic inclusion bodies have been described in early studies in up to 30% of the thalamic nuclei of DM1 patients. Their clinical significance is still unclear. The substantia nigra and the caudate nucleus could be also involved. These inclusions are composed of ubiquitin and microtubule-associated proteins, thus creating the neuropathological substrate for including myotonic dystrophies among degenerative disorders. Thornton demonstrated that mutant RNA accumulates as nuclear *foci* in specific brain areas where muscleblind proteins are also sequestered, leading to deregulated alternative splicing in neurons of specific gene protein including tau, amyloid precursor protein, NMDAR1. The distribution of ribonuclear inclusion was wide. RNA *foci* were also detected in the subcortical white matter and the corpus callosum. Neurofibrillary tangles of the Alzheimer type have been demonstrated in DM1. 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 evidence.

There is limited neuropathological data for DM2. It has been suggested a similar brain tau pathology in DM2 as in DM1, but further studies are needed. (Meola 2007).

Continues from Tab 3. Summary of previous neuroimaging studies results in DMs.

| Author | Year | Reference | Population | Methods | Results |
|---|---|---|---|---|---|
| Meola *et al.* | 1999 | Neurology | 20 DM1, 20 DM2, 20 cntrls | 15 DM1, 17 DM2 MRI; 11 DM1, 10 DM2 $H_2O$ PET; 18 DM1, 12 DM2 Neuropsy | MRI: DM1: 7 cerebral atrophy; 8 WMHLs; DM2: 5 cerebral atrophy, 4 WMHLs. Neuropsy: 50% DM1 and 67% DM2 with impaired visuo-spatial recall; 50% DM1, 33% DM2 with impaired visuo-spatial reconstruction. PET: DM1: more widespread hypoperfusion to the dorsolateral frontal cortex and subcortical regions than DM2; DM2 bilateral decrease in rCBF or orbitofrontal and medial frontal cortex. |
| Martinello *et al.* | 1999 | J Neurol Sci | 5 CDM1 | MRI, Neuropsy | 5/5 with ventriculomegaly (not strictly correlated to cognitive defect); 3/5 with hypoplasic corpus callosum; 2/5 WM abnormalities (mild degree); 3/5 with mild cortical atrophy. |
| Di Costanzo *et al.* | 2001 | Neuroradiol | 20 DM1, 20 asm-cntrls | MRI (T2-relaxometry) | 16/20 with WMHLs (ATWMHLs = 13/20). Widespread T2 prolongation in normal appearing WM (progressing with the course of the disease, muscular disability, brain atrophy). No correlation T2-prolongation - CTG size. Grey matter: trend towards longer T2 (no correlation with other clinical variables). |
| Di Costanzo *et al.* | 2001 | Eur Neurol | 41 DM1, 41 cntrls | MRI | More frequent and severe both dilatation of dilated virchow-Robin spaces and WMHLs in DM1 than cntrls; importance of disease duration. |
| Di Costanzo *et al.* | 2002 | Neuromusc Dis | 5 CDM1, 10 am-ADM1, 10 ddm-ADM1, 20 cntrls | MRI | CDM1: ventriculomegaly, moderate/severe HWMPST (no correlation with age). ADM1: strict correlation to disease duration. Varies from normal findings (except ATWMHLs) in am-pts, to ventriculomegaly and WMHLs in ddm-pts. |
| Di Costanzo *et al.* | 2002 | J Neurol | 66 ADM1 (reviewed) | MRI | Disease duration: positive correlation with WMHLs; negative correlation with Virchow-Robin spaces. 4 subgroups of brain involvement: correlation with disease duration, muscular involvement, WMHLs, brain atrophy. |
| Kassubek *et al.* | 2003 | Neurosc Letters | 10 DM1, 9 DM2, am-cntrls | 3D-MRI | BPF: DM1<DM2<cntrls. No correlation with clinical parameters. Global brain atrophy occurs in both diseases (DM1>DM2). |
| Meola *et al.* | 2003 | Neuromusc Dis | 21 DM1, 19 DM2, 21 cntrls | ECD-SPECT in 5 DM2, Neuropsy | No axis I and II disorders (DM1 and DM2). Avoidant personality trait in DM1 and DM2. impaired frontal functions. No genetic correlation. Frontal and parieto-occipital hypoperfusions in DM2. |
| Kornblum *et al.* | 2004 | J Neurol | 10 DM1, 9 DM2, am-cntrls | MRI | WMHLs and/or brain atrophy in 9/10 DM1 (ATWMHLs in 7/10). WMHLs and/or brain atrophy in 8/9 DM2 (No ATWMHLs). ATWMHLs only in DM1. |
| Antonini *et al.* | 2004 | J Neurol Neurosurg Psy | 22 DM1, 22 asm-cntrls | Voxel Based Morphometry MRI | Global and regional atrophy evaluated. DM1: reduced brain tissue volumes. Grey matter volume inversely correlated with age (DM1>cntrls). No correlation between cortical atrophy and genetic condition or clinical pictures or WMHLs. Cortical atrophy: both frontal lobes, both parietal lobes, both middle temporal gyri, left superior temporal and occipital gyri. |
| Kuo *et al.* | 2005 | Brain & Develop | 2 CDM1, 4 ADM1 | MRI | CDM1: severe mental retardation and HWMPST, ventricular dilatation. ADM1: 2/4 pts with WMHLs in the frontal and/or temporal lobes. |
| Fukuda *et al.* | 2005 | Acta Radiol | 19 DM1, 19 am-cntrls | Conventional and Diffusion-Tensor MRI | Low FA and high MD values in DM1 (NAWM)>cntrls. Low FA and high MD values in WMHLs>NAWM (DM1). Suggestive for microstructural changes in NAWM of DM1. |
| Vielhaber *et al.* | 2006 | Muscle Nerve | 14 DM1, 15 DM2 | Spectroscopic MRI | DM1: reduction of NAA, choline and creatin levels (occipital and temporo-parietal regions; subcortical frontal WM). In DM2: reduction of NAA levels (same regions as DM1). |
| Ota *et al.* | 2006 | Neurosc Letters | 11 DM1, 13 asm-cntrls | Diffusion-tensor and Voxel Based Morphometry MRI | Reduction of FA and increase of MD in subregions of the corpus callosum in DM1, with low volumes in the corresponding areas of the cortex (parietal cortex spared). |
| Giorgio *et al.* | 2006 | J Neurol | 10 DM1, 12 asm-cntrls | MRI | DM1 pts with normal conventional MRI or minimal changes. NBV: DM1 volumes<cntrls. NCV: DM1 volumes<cntrls. |
| Di Costanzo *et al.* | 2008 | Neuromusc Dis | 60 DM1 from 22 families | MRI | Familial aggregation of WMHLs; no relationship with CTG size. |
| Kuo *et al.* | 2008 | Acta Neurol Scand | 17 DM1 | MRI, Neuropsy | Correlation between temporo-insular lesions and intelligence tests. Correlation between frontal WMHLs and aging. |

Abbreviations: **pt(s)**: patient(s), **cntrls**: controls; **am-cntrls**: age-matched controls; **sm-cntrls**: sex-matched controls; **asm-cntrls**: age and sex- matched controls; **pDM1**: paternally inherited DM1; **mDM1**: maternally inherited DM1; **MS**: multiple sclerosis; **CDM1**: congenital DM1; **ADM1**: adult onset DM1; **ddm-ADM1**; disease duration-matched DM1; **Neuropsy**: neuropsychological tests; **FDG**: fluoro-deoxyglucose; **ECD**: 99mTc-ethylcysteinate dimer; **MMSE**: mini-mental state examination; **WMHLs**: White Matter Hyperintense Lesions; **ATWMHLs**: Anterior Temporal White Matter Hyperintense Lesions; **(r)CMRGlu**: (Regional) Cerebral Glucose Metabolic Rates; **I.Q.**: Intelligence Quotient; **WM**: White Matter; **NAWM**: Normal-Appearing White Matter; **HWMPST**: Hyperintensity of White Matter Postero-Superior to Trigones; **BPF**: Brain Parenchymal Fraction; **FA**: Fractional Anisotropy; **MD**: Mean Diffusivity; **NAA**: N-acetylaspartate; **NBV**: Normalized Brain Volumes, **NCV**: Normalized Cortical Volumes.

# AIM OF THE STUDY

Aim of the present study is to establish, by brain MRI, the recurrence, localization, patterns of distribution of white matter hyperintense lesions in a cohort of DM1 and DM2 patients, and to compare the results with SPECT perfusion imaging patterns, neuromuscular and neuropsychological profiles.

# MATERIALS AND METHODS

## *PATIENTS:*

### DM1:

We recruited 50 DM1 patients (32 males, 18 females; mean age: 40 years, with age ranging from 12 to 73 years) in our Neuromuscular Unit (Department of Neurosciences, School of Medicine, University of Padova, Italy). All DM1 patients were molecularly determined and subgrouped on the basis of [CTG]n expansion size as follows: E1 (<150 CTG; 14%), E2 (150-1000 CTG; 67%), E3 (>1000 CTG; 19%) (The International Myotonic Dystrophy Consortium, 2001) (Fig. 23). The patients were further stratified into three groups as follows: CTG<500; CTG 500-1000; CTG>1000. Each patient was tested manually for muscle strength using the five-point MRC scale, and severity of muscular involvement was scored by the muscular impairment rating scale (MIRS) (Mathieu 2001) (Tab. 4). Age of disease onset of the patients ranged from 0 to 66 years (mean 22.1 ± 16.9 years), with an estimated disease duration of 18.1 ± 10.2 years. Mean educational level was 10 ± 4 years (range 4-18).

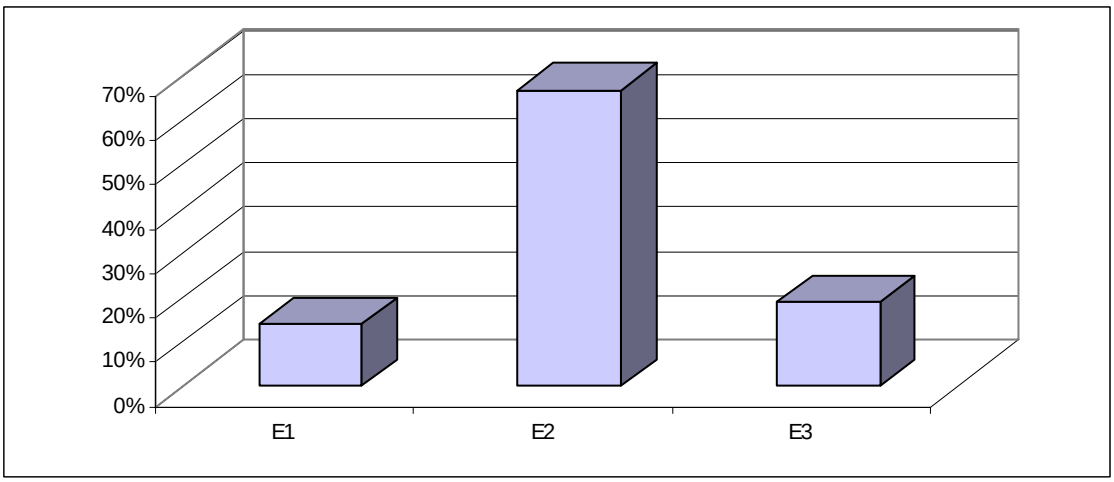



**Fig. 23.** Distribution of DM1 patients, on the basis of the E-class (CTG triplet expansion size).

Table 4. Muscular Impairment Rating Scale (MIRS)

| Grade | Description |
|---|---|
| I | No muscular impairment |
| II | Minimal signs |
| | myotonia, jaw and temporal wasting, facial weakness, neck flexor weakness, ptosis, nasal speech, no distal weakness except isolated digit flexor weakness |
| III | Distal weakness |
| | no proximal weakness except isolated elbow extensor weakness |
| IV | Mild to moderate proximal weakness |
| V | Severe proximal weakness (MRC#-3/5) |

## DM2:

Fourteen DM2 patients (5 males, 9 females; mean age: 53.5 years, with age ranging from 28 to 71 years) were molecularly characterized by the detection of a [CCTG]n expansion in the first intron of ZNF9 gene on chromosome 3q21 (Liquori 2001). Muscular involvement was scored by MIRS (Mathieu 2001). Age of disease onset ranged from 5 to 67 years (mean 37.6 ± 19.2 years), with an estimated disease duration of 16.3 ± 10.2 years. Mean educational level was 10 ± 5 years (range 4-18).

None of the selected subjects presented a clinical history of cerebrovascular or psychiatric disorder, multiple sclerosis, brain hypoxic insult, meningoencephalitis, major endocrinological diseases (such as thyreotoxicosis or diabetes), malignancies, or other neurological diseases affecting muscle or cognitive functions; alcohol or drug abuse were further exclusion criteria.

*MRI STUDY*

Brain MRI scans were obtained in 46/50 DM1 (29 males, 17 females, mean age 39.8 ± 15.9 years) and in 12/14 DM2 patients (5 males, 7 females, mean age 52.7 ± 14.8 years). Three DM1 and 1 DM2 patient denied to participate; 1 DM1 patient had deceased; 1 DM2 patient had a cardiac pacemaker. MRI studies were performed with 1.0-T magnetic (Marconi Picker Polaris 1.0). Axial and sagittal spin-echo T1-weighted images (531/12/2 [TR/ TE/ NEX]), axial and coronal FSE double echo DP/T2 images (3523/20-120/2/4, [TR/ TE/ NEX/ ETL]), axial FSE FLAIR (6000/80/2/2100/4, [TR/ TE/ NEX/ TI/ ETL]) and axial diffusion-weighted echo planar images (6597/ 113.4/1 [TR/ TE/ NEX]; b-value 800 s/mm$^2$, matrix 128x128) were obtained. The MRI studies focused on white matter lesions. White matter abnormalities were graded, on T2/FLAIR images, according to the age related white matter change score (ARWMC score: 0=absent; 1=focal; 2=initially confluent; 3=diffuse involvement in the frontal, parieto-occipital, infratentorial, temporal, insular regions; and 0=absent; 1=one focal lesion greater than 5 mm; 2=more than one lesion; 3=confluent; in the basal ganglia) (Wahlund 2001, Table 5 and Table 6). Both hemispheres were scored. An ARWMC score with a 3 mm-diameter lesion cut-off was also attempted, in order to improve the detection of abnormalities in brain MRI scans.

**Table 5.** ARWMC score for supratentorial lesions

| Score | Kind of lesion |
|---|---|
| 0 | No lesion |
| 1 | Focal lesions with > di 5mm diameter |
| 2 | Initially confluent lesions |
| 3 | Diffuse involvement |

**Table 6.** ARWMC score for infratentorial lesions

| Score | Kind of lesion |
|---|---|
| 0 | No lesion |
| 1 | One focal lesions with > di 5mm diameter |
| 2 | More than one lesion |
| 3 | Confluent lesions |

## *SPECT STUDY*

Single photon emission computed tomography (SPECT) imaging of cerebral blood flow was performed in 21 DM1 patients (12 males and 9 females, mean age 37 years, 17 of whom were also studied by MRI) and in 9 DM2 patients (3 males and 6 females, mean age 54 years, 7 of whom were also studied by MRI). Normal diet and medication were maintained, with no specific patient preparation. Pregnancy was the only contraindication to this investigation and none of our patients was pregnant. To minimize the harmful stimuli, each patient received 740 MBq of $^{99m}$Tc ethyl-cysteinate dimer (ECD - *Neurolite*®) intravenously via a butterfly needle placed 10 minutes beforehand. The tracer was injected with patients in the supine position, awake and in the resting state, in a dimly light room. Forty-five minutes after injection of the tracer, images were acquired using a triple-head gamma camera (PHILIPS-IRIX) with low energy ultra high resolution collimators (LEUHR). The images were acquired on a 360° orbit ('step and shoot', 128 x 128 matrix zoom factor 1.6, 3°/step) with a 30-second step. Iterative reconstruction was performed and the resulting transaxial slices were filtered by a low-pass filter. Complete tri-dimensional reconstruction was obtained from single transverse, coronal and sagittal images. Regional uptake was analysed by a commercial package (*Neurogam*®) normalizing the volume in the Talairach's space and using the cerebellum as reference area (Fig. 24). Voxel per voxel tracer uptake computed analysis was bilaterally done to test frontal, temporal, parietal lobes and seven specific Brodmann's areas in both hemispheres: area 7 (somatosensory association cortex, in the superior parietal lobe, involved in a variety of spatial transformations such as mental rotation and visuo-motor coordination); area 9 (dorsolateral prefrontal cortex, involved in episodic long-term memory, working

memory, sustained attention, responses inhibition); area 10 (rostral prefrontal cortex area, plays an important role in strategic processing of memory retrieval, prospective memory, context memory, in the maintenance and realization of delayed intentions); area 19 (tertiary visual association cortex, involved in feature extraction, shape recognition and visual attention); area 24 (ventral anterior cingulated cortex, involved in motivation, will and cognitive control such as in interference tasks); area 28 (posterior entorinal cortex, represents a relais in memory consolidation; particularly contributes to object-in-place scene memory); area 38 (temporopolar area, main area of identity representation, both at semantic level -left- and at autobiographic level -right) (Harris 2000, Lloyd 2000, Ranganath 2003, Marklund 2007, Burgess 2007, Okuda 2007, Carter 2007, Charles 2004, Noulhiane 2007)**.** The decision to test the Brodmann's areas was based on both the neuroimaging literature on DMs and on our previous retrospective evaluation of brain perfusion patterns in DM1. (Figures 25 and 26).

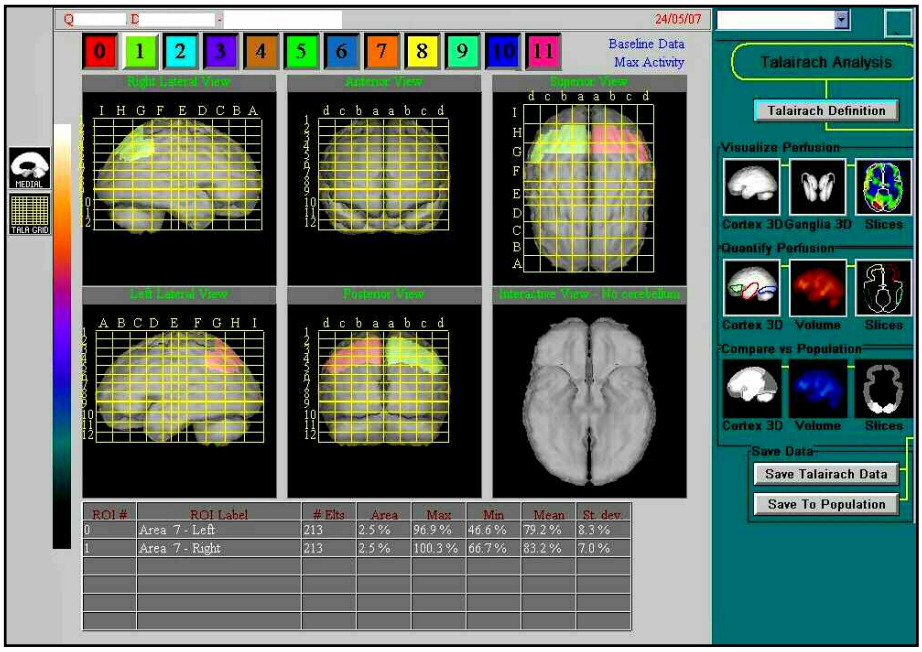



**Fig. 24.** The picture shows the main interface window of *Neurogam* software. In the principal panels, six rotational models of 3D brains are shown. The volume normalization was calculated in the Talairach's space using the cerebellum as reference area.

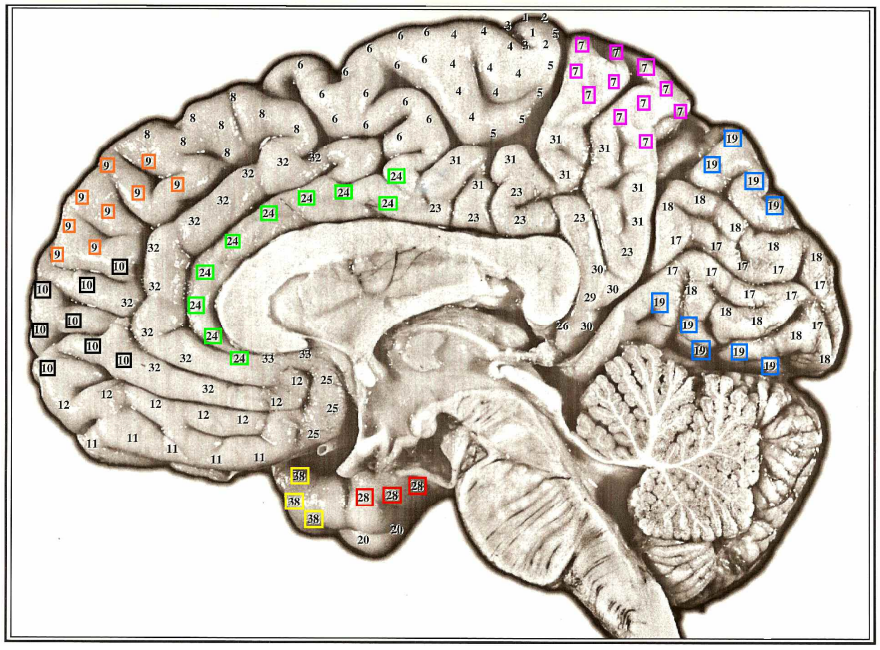


**Fig. 25.** Sagiptal median section of the brain. Brodmann's areas are indicated. The picture shows 7 coloured areas of interest: area 9 in orange, area 10 in black, area 24 in green, area 38 in yellow, area 28 in red, area 19 in blue, area 7 in pink. (Modified from J.J. Warner's *Atlas of Neuroanatomy*).

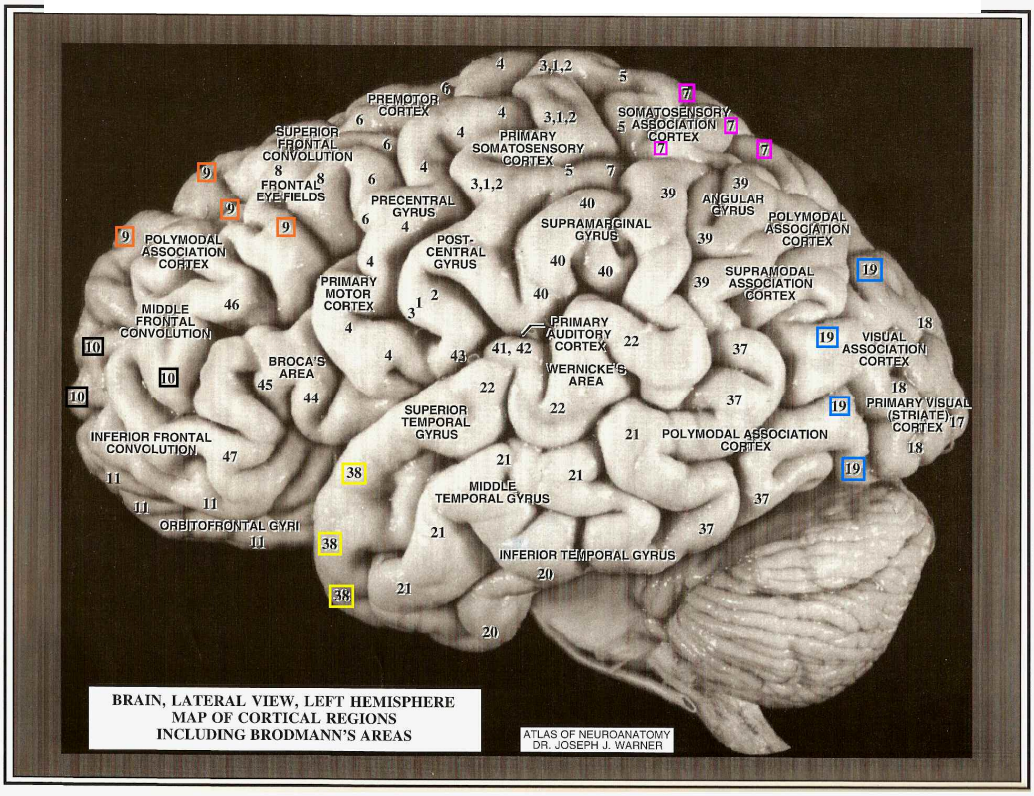



**Fig. 26**. Left hemisphere external sight: map of the cortical regions and Brodmann's areas. Area 9 (orange), 10 (black), 38 (yellow), 19 (blue) and 7 (pink) are indicated. (Modified from J.J. Warner's *Atlas of Neuroanatomy*).

### *NEUROPSYCHOLOGICAL ASSESSMENT*

Cognitive assessment was performed in 48 DM1 (30 males, 18 females, mean age 40.1 ± 15.1 years) and 9 DM2 patients (3 males, 6 females, mean age 54.8 ± 13.8 years); 44 age-matched control subjects were also evaluated (30 males, 14 females, mean age 37.0 ± 15.1 years, age range 10-70, educational level 10 ± 3 years.). In order to assess non verbal intelligence we performed Raven's pogressive Matrices (PM47; I.Q. calculated for global intelligence); we administered the Stroop (Word, Colour, Colour-Word) and Fluency tests to assess frontal executive fuctions; the Wechsler Memory Scale and Corsi's Block tests to evaluate memory and learning functions; the Rey-Osterrieth Complex Figure (Copy and Memory) to test visuo-spatial abilities.

### *STATISTICAL ANALYSIS*

Student's t test was performed for normally distributed variables. The Mann Whitney U and Wilcoxon tests were used for ordinal variables, while Pearson's $\chi^2$ was calculated for categorical variables. Spearman's Rho was used to test the significance of a linear correlation between two variables when at least one was ordinal. The significance level was set at $p<0.05$.

# RESULTS

*MRI:*

DM1:

Nine patients (19.5%; C.I. 95%: 8% - 31%) had normal MRI neuroimaging. Basal ganglia were affected in two cases (2/46; 4.4%) and infratentorial structures in three cases (3/46; 6.5%). No side-to-side difference was observed (Wilcoxon test). T2/FLAIR showed supratentorial, bilateral, symmetrical focal or diffuse white matter abnormalities in 37/46 DM1 (80.4%). Diffuse symmetrical WMHLs were present in the insular regions in 28 DM1 patients (60.9%) and in 18 of them, a peculiar subcortical symmetrical diffuse involvement of the polar-temporal regions was also detected (Fig 25). One patient had an isolated temporal involvement. While focal and/or initially confluent WMHLs were detected in the fronto-parieto-occipital lobes, lesions were absent or diffuse in the temporo-insular lobes (Figures 25, 30 and 31; Figure 33, panel A). MRI documented >8mm thickening of the skull in 64.4% of the patients (Figures 26 and 27), paranasal sinuses hypertrophy in 74% (Figures 28 and 29), cortical atrophy and ventriculomegaly in 30,4%. Frontal and parieto-occipital involvement strongly correlated with poorer performance in Rey M and Corsi's tests, while temporo-insular WMHLs did not significantly correlate with neuropsychological test performance (Table 7). Major intellectual impairment was associated with more diffuse morphological abnormalities detected by MRI. 75% of the patients with CTG>1000 had abnormal frontal MRI, while only 25% of those with CTG<500 showed frontal abnormalities. A trend towards more severe central involvement in patients harbouring larger expansions was also observed in other cerebral territories, without reaching

significance (Table 8). This significance was lost when E1, E2, E3 classes of expansion were used to stratify the DM1 population. The total lesion load appeared greater in the older DM1 patients than in the younger ones, with a linear correlation (p=0.0001), likely due to an age-related effect. A significant difference was found in total lesion load between DM1 aged <40 years and DM1 aged >40 years (p=0.005).

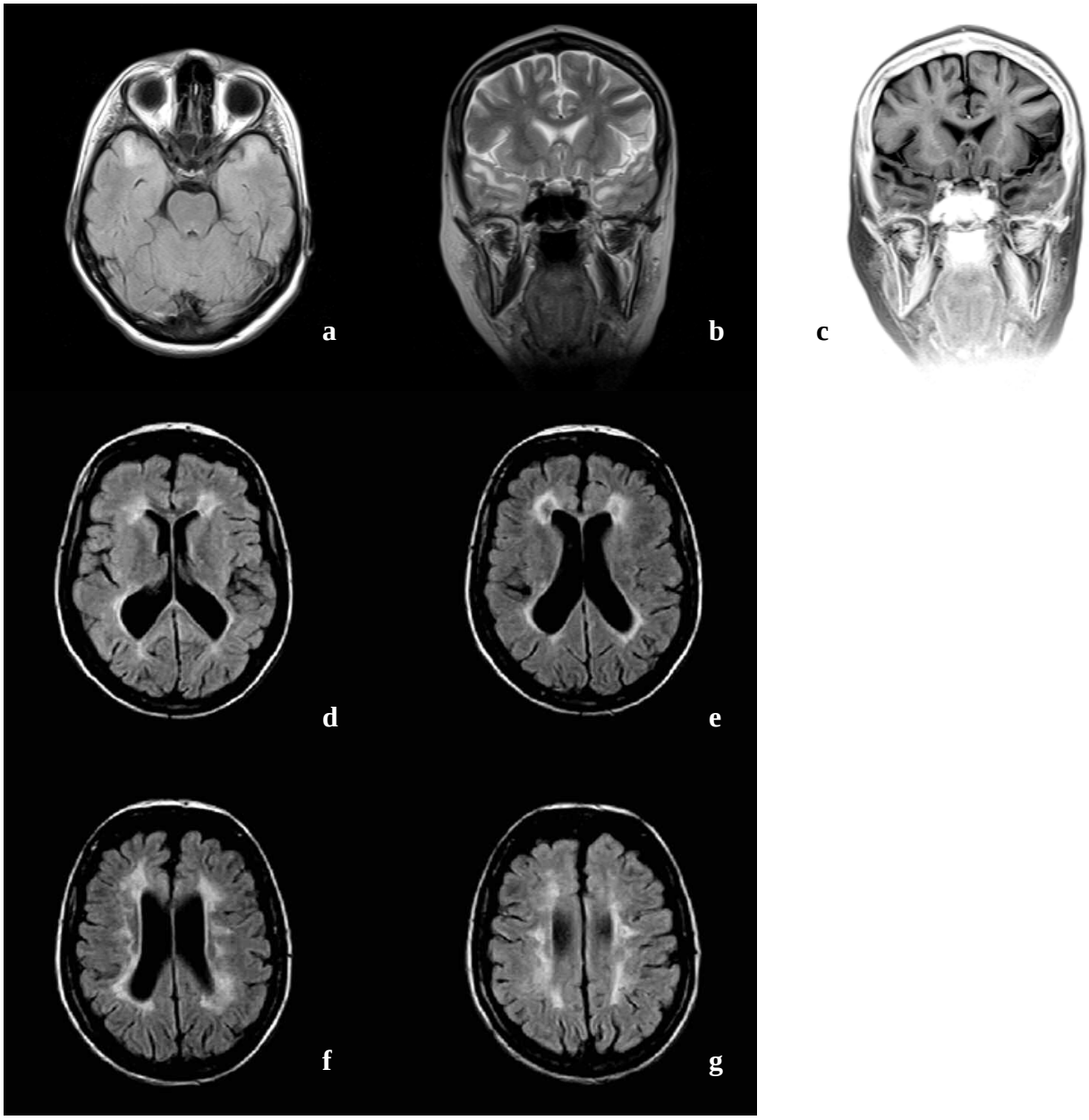



**Fig. 25.** MRI (T2/FLAIR) in DM1. In panel *a* (axial) and *b* (coronal): diffuse subcortical abnormalities (WMHL) are bilaterally detected in the polar temporal regions. In panel *c* MRI coronal T2 scan inverted image highlights the white matter changes in the temporal poles. In panel *d* to *g*: several diffuse subcortical abnormalities are observed throughout the white matter of the fronto-parieto-occipital planes in a severe case of DM1.

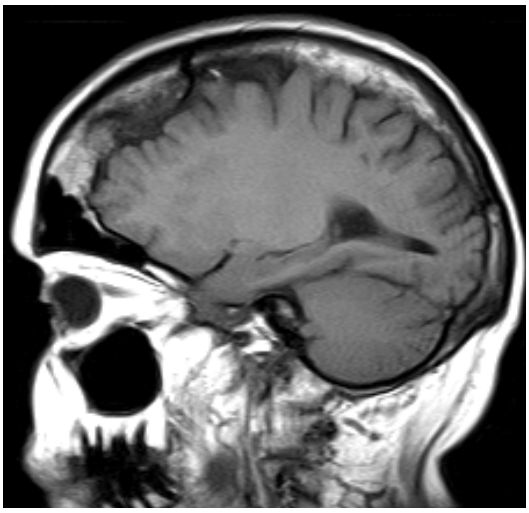


**Fig. 26.** T1-weighted sagiptal scan. The image shows a clear thickening of a DM1 patient's skull.

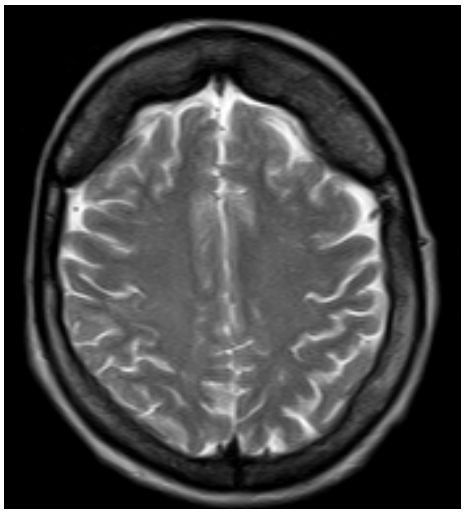


**Fig. 27.** T2-weighted axial scan. Marked thickening of a DM1 patient's skull is shown.

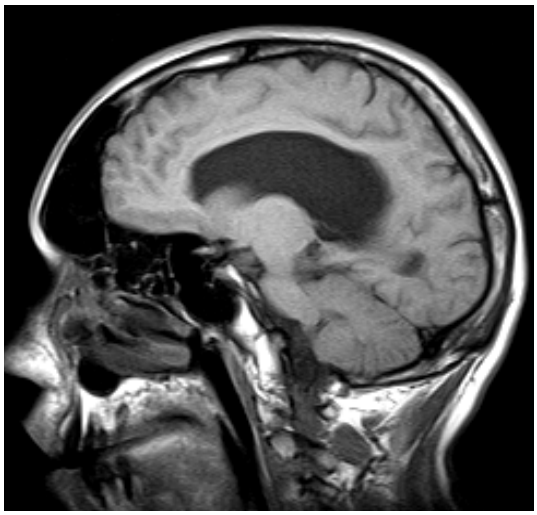


**Fig. 28.** T1-weighted sagiptal section of a DM1 brain. Paranasal sinuses are globally enlarged.

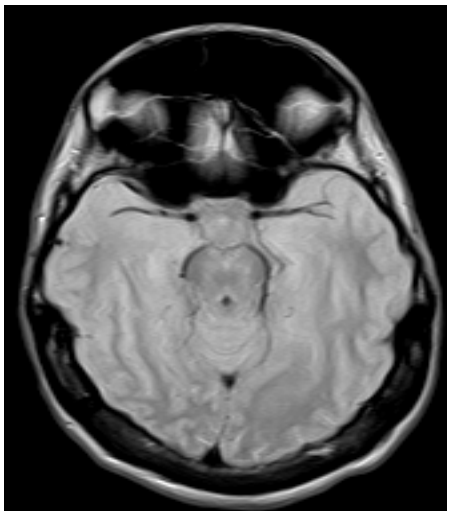


**Fig. 29.** DP-weighted axial section of a DM1 brain. Frontal, sphenoidal and etmoidal sinuses are markedly dilatated.

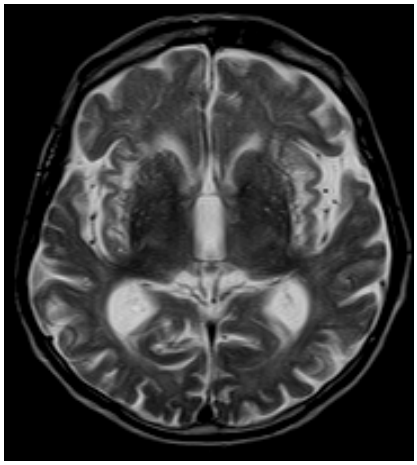


**Fig. 30.** Axial T2-weighted scan showing a diffuse involvement of the white matter in both insular regions.

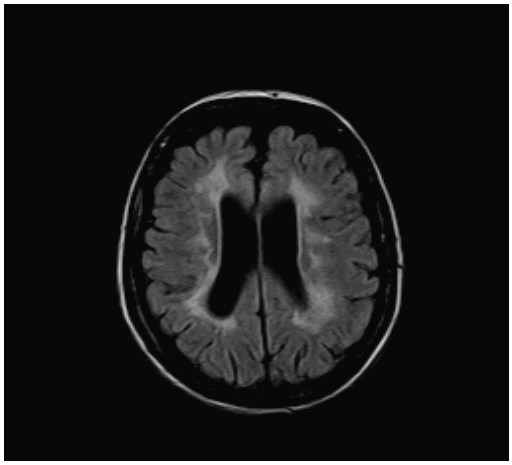


**Fig. 31.** Axial FLAIR scan. Diffuse abnormality of the periventricular white matter.

*Table 7. Relationship between brain areas involved by WMHLs and neuro-psychological performances in the DM1 cohort.*

| Brain areas | Test | *p* |
|---|---|---|
| ***Frontal*** (i= 21, ni= 22) | Stroop CW | 0,04 |
| | Rey C | 0,02 |
| | Rey M | < 0,001 |
| | Corsi | 0,01 |
| ***Parieto-Occipital*** (i= 24, ni= 19) | Stroop CW | 0,04 |
| | Rey M | < 0,001 |
| | Corsi | 0,03 |
| ***Insular*** (i= 26, ni= 17) | Rey M | 0,03 |
| ***Temporal*** (i= 18, ni= 25) | All tests | n.s. |

i= involved; ni= not involved

n.s. = not significative

*Table 8. Relationship between CTG expansion size and frontal lobe involvement in MRI**

| | **frontal MRI** | | |
|---|---|---|---|
| **CTG expansion** | **normal** | **pathologic** | **Total** |
| <500 CTG | 12 pts | 4 pts | 16 pts |
| | 75,00% | 25,00% | |
| 500-1000 CTG | 4 pts | 8 pts | 12 pts |
| | 33,00% | 67,00% | |
| >1000 CTG | 2 pts | 6 pts | 8 pts |
| | 25,00% | 75,00% | |
| Total | 18 pts | 18 pts | 36 pts |

pts = patients

χ2 Pearson: 7.33. *p*=0.026

(*) CTG-MRI match was done only in those cases with a time delay <2 years between the two methods.

DM2:

MRI was normal in 2/12 patients (16.7%). Signal abnormalities were documented in 10 DM2 patients. Basal ganglia were affected in 3 cases, infratentorial structures in one. Nine patients had MRI changes in frontal, parietal and/or occipital lobes (Fig. 32). Such abnormalities were scattered focal, confluent or diffuse. No temporal or insular involvement was observed in any patient (Figure 33, panel B). No significant asymmetrical distribution was seen in DM2 brains (Wilcoxon test). Statistics did not highlight any significant correlation between MRI and neuropsychological test results.

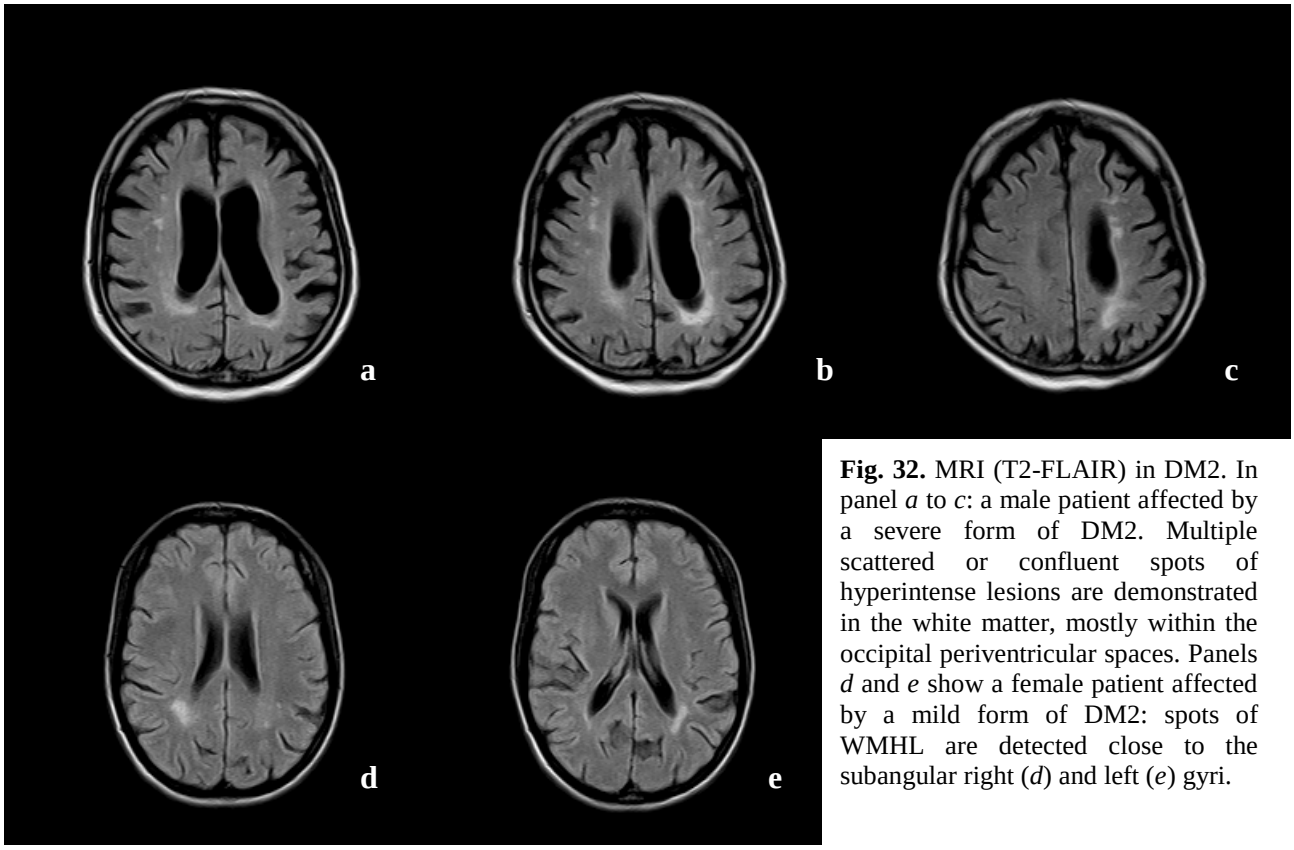



**Fig. 32.** MRI (T2-FLAIR) in DM2. In panel *a* to *c*: a male patient affected by a severe form of DM2. Multiple scattered or confluent spots of hyperintense lesions are demonstrated in the white matter, mostly within the occipital periventricular spaces. Panels *d* and *e* show a female patient affected by a mild form of DM2: spots of WMHL are detected close to the subangular right (*d*) and left (*e*) gyri.

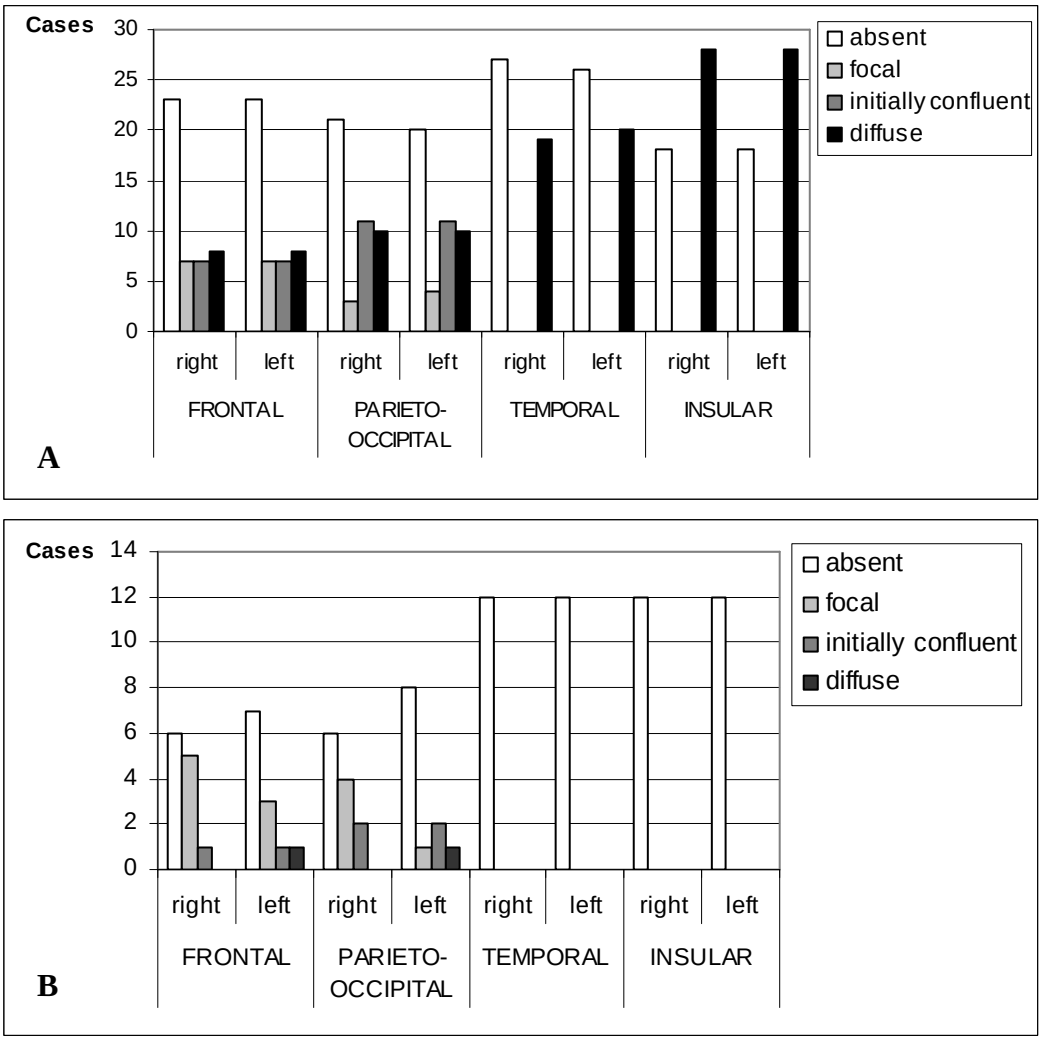



**Fig. 33.** The DM1 and DM2 cohorts are shown in panels A and B, respectively. Distribution of WMHLs detected by MRI and classified using the ARWMC score. The four WMHLs (absent, focal, initially confluent, diffuse) are indicated by different colours. Four main areas of interest are shown: frontal, parieto-occipital, temporal, insular area. Both left and right hemispheres are considered. Panel A shows, in DM1, focal and initially confluent lesions in frontal and parieto-occipital regions. Only absent/diffuse lesions are seen in temporo-insular regions. Panel B shows, in DM2, focal and initially confluent lesions in frontal and parieto-occipital regions only, while the temporo-insular regions are intact.

### *COMPARISON DM1/DM2:*

Mann-Whitney's U test revealed a significant difference in temporal and insular involvement between DM1 and DM2. The WMHLs burden was significantly higher in DM1 compared to DM2 patients (Table 9). When ARWMC were scored using a 3 mm-diameter lesion cut-off, no significant differences were detected.

*Table 9. Mann-Whitney U test conducted on DM1/DM2 cohorts: measure of the mean ARWMC involvement documented by MRI.*

| MRI | ARWMC scores (± s.d.) | | *p* |
|---|---|---|---|
| | DM1 | DM2 | |
| Frontal | 1.00 ± 1.18 | 0.63 ± 0.77 | n.s. |
| Parieto-occipital | 1.23 ± 1.25 | 0.67 ± 0.89 | n.s. |
| Infra-tentorial | 0.07 ± 0.25 | 0.08 ± 0.29 | n.s. |
| Temporal | 1.27 ± 1.48 | 0.00 ± 0.00 | 0.02 |
| Insular | 1.83 ± 1.48 | 0.00 ± 0.00 | <0.01 |
| Basal ganglia | 0.09 ± 0.47 | 0.33 ± 0.69 | n.s. |
| Total | 0.90 ± 0.73 | 0.28 ± 0.31 | 0.01 |

n.s. = not significative

## *SPECT IN DM1/DM2*

A normal brain perfusion pattern was seen in 4/21 (19%) DM1 patients. A mild reduction of perfusion, almost exclusively involving the left hemisphere (mainly parieto-temporo-occipital lobes), was documented in 17 patients; of these, 7 patients showed very mild right parieto-temporal hypoperfusion (Fig. 34, panel A). Semiquantitative analysis of regional perfusion by *Neurogam*® showed significant hypoperfusion in the left compared to the right area 7 ($p=0.01$). Right-side hypoperfusion was detected in area 28 ($p<0.0001$). A similar asymmetrical pattern of perfusion in the seven considered areas was also observed within the DM2 cohort (Fig. 35). In DM2, a mild reduction of perfusion was observed with a qualitative analysis approach in 7/9 patients. In particular, the most significant hypoperfusions were detected in the posterior planes of the parietal lobes (severe left parietal hypoperfusion in 1 patient, mild left parieto-temporal involvement in 2 cases; very mild bilateral parietal involvement in 4 patients) (Fig. 34, panel B).

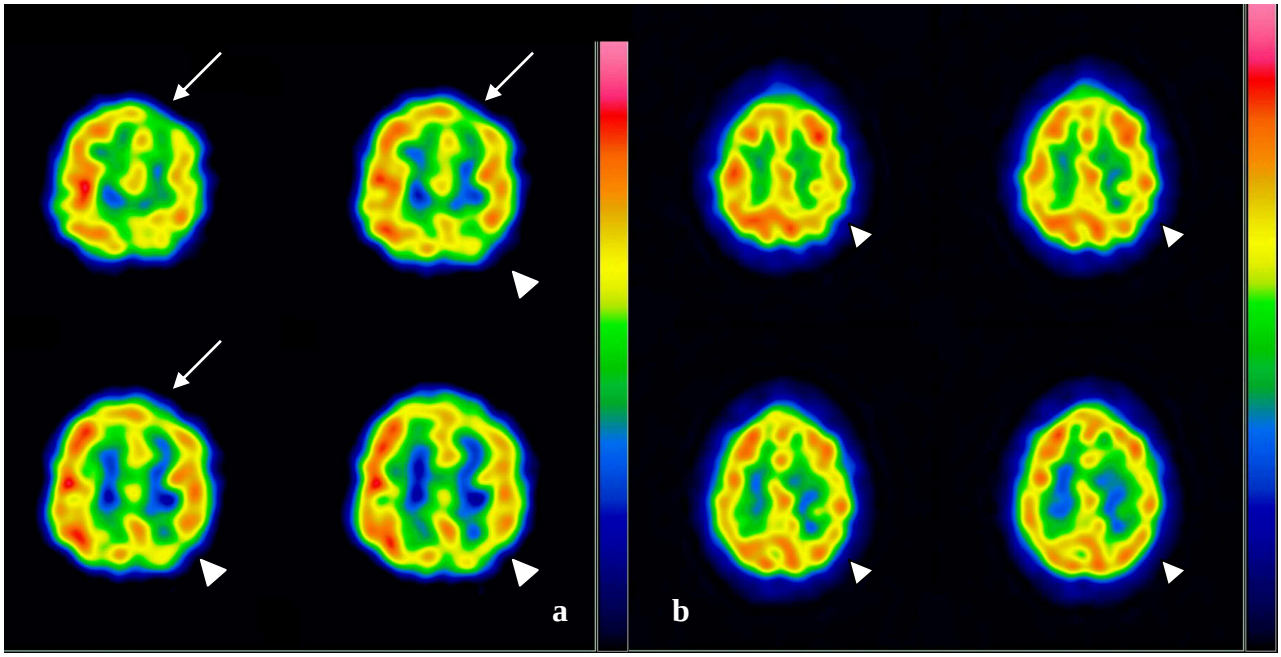



**Fig. 34.** Brain axial SPECT in DM1 and DM2. Panel *a* shows diffuse inhomogeneous hypoperfusion in left hemisphere, documented by reduced tracer-uptake throughout frontal (arrows) and parieto-occipital lobes (arrow-heads) in DM1. Panel *b* shows a very slightly reduced tracer-uptake in the left hemisphere, almost exclusively detectable within the superior planes of the parietal lobe (arrow-heads) in a DM2 patient.

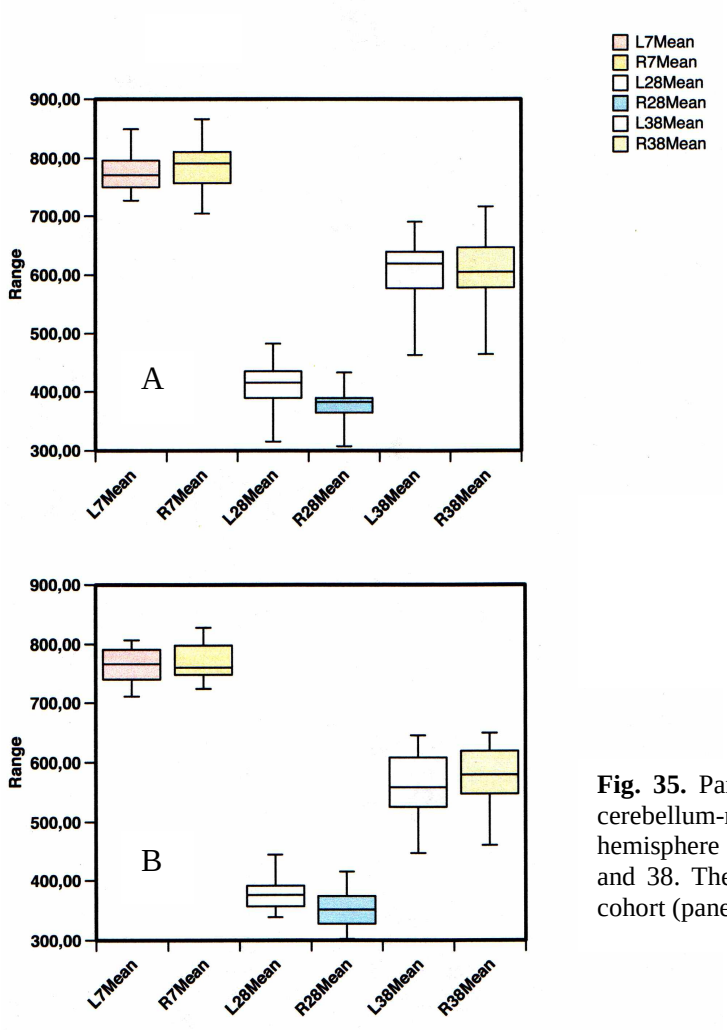



**Fig. 35.** Panel A shows the comparison between the cerebellum-normalized flow-rate of left and right hemisphere in DM1 patients, in Brodmann's areas 7, 28 and 38. The same comparison is performed in a DM2 cohort (panel B).

### *NEUROPSYCOLOGICAL RESULTS IN DM1/DM2/CONTROLS*

Greater variability in neuropsychological testing was observed in the DM1 compared to the DM2 cohort. Executive dysfunction was frequently seen (by the Stroop and phonemic fluency tests). Mann-Whitney's U test showed a significant difference between DM1 and DM2 patients in Rey-Osterrieth's Complex Figure Copy and in calculated I.Q. (p=0.04). (Table 10).

Table 10. Neuropsychological tests results: comparison between DM1 and DM2 (Mann-Whitney)

| MRI | Mean values ± s.d. | | *p* |
|---|---|---|---|
| | DM1 | DM2 | |
| Stroop W | 54.0 ± 27.5 | 53.1 ± 13.5 | n.s. |
| Stroop C | 81.8 ± 30.7 | 93.6 ± 26.3 | n.s. |
| Stroop CW | 179.8 ± 62.3 | 200.3 ± 80.6 | n.s. |
| Semantic Fluency | 15.5 ± 5.0 | 13.5 ± 3.5 | n.s. |
| Phonemic Fluency | 9.2 ± 4.1 | 9.6 ± 4.5 | n.s. |
| Rey Copy | 29.4 ± 7.1 | 31.8 ± 7.4 | 0.04 |
| Rey Memory | 19.1 ± 8.1 | 17.6 ± 9.9 | n.s. |
| PM47 | 30.6 ± 5.1 | 33.1 ± 3.2 | n.s. |
| QI calc. | 98.8 ± 14.0 | 107.8 ± 9.1 | 0.04 |
| WM score | 101.6 ± 14.1 | 105.3 ± 13.0 | n.s. |
| QM | 108.9 ± 21.7 | 114.4 ± 22.1 | n.s. |

n.s. = not significative.

### *NEUROMUSCULAR ASSESSMENT AND MRI*

The MIRS score was used to assess neuromuscular impairment in our DM1 cohort. MIRS scores varied between 1 and 5 (mild to severe impairment). Four patients scored 1, 10 scored 2, 16 scored 3, 18 scored 4, 2 scored 5 (mean value 3.1 ± 1.0). Of the 14 DM2 patients, 8 scored 1, 0 scored 2, 1 scored 3, 4 scored 4, 1 scored 5 (mean value 2.3 ± 1.6). A correlation between age of disease onset and MIRS was attempted. No significance was seen in DM1 ($p=0.52$), while a slight correlation was documented in DM2 ($p=0.018$). There was no significant correlation between MIRS and disease duration, in either DM1 or DM2. MIRS and CTG expansion size were slightly correlated in DM1 ($p<0.001$). No significant correlation was found between MIRS and total lesion load of WMHLs on MRI in either cohort.

## DISCUSSION

Brain involvement in myotonic dystrophies has been a subject of debate in the literature (Table 3), particularly in recent years, with the introduction of new brain imaging techniques (spectroscopy, voxel based morphometry,…), although there are some methodological discrepancies. According to a recent review, some results appear to be partially contradictory (Meola 2007). The most frequently performed investigation was MRI, which gave interesting results for both proper evaluation of extent of cortical atrophy and white matter involvement. One characteristic specific to imaging in DM1 seems to be the changes within the temporal poles and the insulae, detectable in many of these patients. However, the nature of such alterations and their clinical implications are not clear. Both metabolic and perfusion PET studies have been performed in DM1, suggesting focal perfusion abnormalities and changes in brain glucose metabolism (Fiorelli 1992, Mielke 1993, Annane 1998, Meola 1999). Nonetheless, it is unclear whether these phenomena are due to a defect in vascular extraction or in membrane flux of the adopted tracers ('membrane disease' interpretation). SPECT perfusion studies are very few (Chang 1993, Meola 2003) and indicate the presence of focal hypoperfusion of the brain cortex, in some cases asymmetrical, variable in degree, with prominent frontal localization. The small sample size of patients studied does not permit the results to be generalized to the whole DM1 population, being typically very heterogeneous. Besides, there is a lack of studies aimed at investigating brain involvement in DM2 by MRI (Hund 1997, Meola 1999, Kassubek 2003, Kornblum 2004, Vielhaber 2006). White matter changes or atrophy were documented in DM2 as well as in DM1, with variable extent, distribution and relationships with clinical parameters, thus demonstrating that

brain involvement also occurs in DM2. Likewise, the few works with PET or SPECT perfusion studies, in DM2 as well as in DM1 patients, have permitted detection of spots of focally reduced cortical brain perfusion (Meola 1999, Meola 2003). Studies on cognitive and personality patterns of DMs deserve special mention. Finding one proper classification for the various cognitive patterns of DM1 has clearly always been very difficult, because of the great variability of DM1. Despite this, avoidant, obsessive-compulsive, passive-aggressive and schizotypic personality have been indicated as the traits that best fit the 'average' DM1 patient's features (Meola 2007). Many neuropsychological studies have reported deficits in executive functions and poorer visuo-spatial performances in DM1. Neuropsychological testing batteries might not be sufficiently disease-focused: new more probing neuropsychological studies are needed on larger, well-stratified DM1 samples. There is also a lack of studies on large samples of DM2 patients. The few available ones suggest that DM2 have similar but less severe profiles compared to DM1 (Meola 1999, Meola 2003). Hence, such preliminary results indicate that there are parallels between cognitive and instrumental neuroimaging findings. In our study we recruited a DM1 and a DM2 cohort; both were subjected to MRI, SPECT and a neuropsychological battery. MRI revealed a high frequency of white matter abnormalities in patients affected by DM1 (80.5%). A subgroup of these (48.6 %) showed peculiar polar-temporal and insular changes, which were diffuse and almost symmetrical and seemed to be very specific for DM1 (in fact, no similar cases were found in DM2). The CTG triplet was generally much more expanded in the case of diffuse subcortical involvement, especially in the fontal lobe. Patients with typical temporal involvement were not characterized by any specific neuropsychological pattern, but it is reasonable to hypothesize that future studies with specific in-depth

associative fronto-temporal tests will more clearly establish the functional relapses of such brain damage. In general, DM1 patients with major and global white matter involvement also showed more severe cognitive defects. We could hypothesize that at least two kinds of lesions may coexist in DM1 patients: polar-temporal/insular lesions (or ATWMHLs) and non-ATWMHLs. The ATWMHLs are an *all-or-nothing* phenomenon. They could be conceived as a congenital hallmark of cortical dysplasia, possibly manifesting in the early stages of embryonal development, and subsequently persisting in adult life as a unique residue of brain dysembryogenesis, even in patients with good cognitive performance. Another interpretation considers DM1 as a pro-geric disease: non-ATWMHLs being instead conceived as an age-related effect on DM brains. In congenital myotonic dystrophy, a dysregulated maturation of muscle fibres from myotubes/myoblasts has been suggested as a possible pathogenic mechanism, where toxic accumulation of endonuclear RNA-triplets causes aberrant splicing and abnormal behaviour of proteins involved in the early stages of muscle development. A similar pathogenic mechanism, expressed during fetal life, could determine an abnormal migration and/or maturation of cortical neurons, thus remaining as a maturative defect, with few or no consequences on cognitive profile. A likely congenital hallmark (ATWMHL) in the brains of adult-DM1 patients could represent a sort of link between congenital DM1 and adult-onset DM1. Hence, an old trace of developmental disturbances may be left in the brains of adult patients. SPECT investigation, aimed at evaluating brain perfusion in specific functional areas with a dedicated software, has generally documented changes of mild degree. Yet, these changes seem to confirm slightly predominant left hemispheric involvement. Such data might not be compared to MRI results, since MRI scans are specifically set for analysis of subcortical regions,

where the main abnormalities were actually documented. Moreover, the observed trend of left-right asymmetrical perfusion was quite similar in the DM1/DM2 cohorts, as in aging. This hypothesis is supported by observation of a similar pattern of asymmetrical brain perfusion in a group of 13 non-DM1/DM2 subjects, previously subjected to SPECT. Asymmetric perfusion of the brain is influenced by aging, with some dependance on gender. Favourable global perfusion at the right hemisphere has been observed in normal brains (especially in the orbito-frontal cortex, superior temporal gyrus, caudate nucleus). Moreover, side asymmetry seems to increase with age. This age-related effect appears to be more relevant in the left prefrontal and lateral frontal cortex, superior temporal and insular regions. Paradoxically, the right infero-medial temporal cortex shows increased activity with aging (Van Laere 2001). Besides, FDG-PET studies have demonstrated a reduction of frontal cortex metabolism with aging and highlight the presence of a constant asymmetric activity (right more than left) in the temporal lobes (Loessner 1995). Therefore, as far as DM1 is concerned, our study confirms the data from previous studies on brain involvement with white matter changes. Conversely, the size of our DM1 cohort permitted closer evaluation of the frequency, extent, distribution and clinical relapses of this involvement. Cases with larger CTG sizes had diffuse white matter abnormalities (especially in the frontal lobes) and higher MIRS scores. By contrast, there was no correlation between MRI lesion loads and muscular impairment. Molecular instability, peculiar to this disease, might explain the lack of linear correlation between these parameters. Just as molecular mosaicism displays variable trinucleotide expansions in different tissues in the same patient, so we could argue that the clinical phenotype might be characterized by different degrees of involvement in several tissues, organs and systems. This '*clinical*

*mosaicism*' seems to more appropriately describe the wide DM1 phenotypic spectrum. With regard to the DM2 cohort, MRI has again been proved valuable in detecting the presence of abnormalities within the subcortical white matter, but the distribution of these lesions seems more asymmetrical in some cases and, in general, less extensive and diffuse compared to the DM1 cohort. Interestingly, the presence of WMHLs was not documented in the polar-temporal or insular regions in our DM2 cohort. No correlation was found between cognitive involvement (mostly of mild degree) and number or distribution of WMHLs, nor between cognitive profile and SPECT. Only a slight impairment of constructive visuo-spatial functions with anomalies of parietal perfusion was observed.

# CONCLUSIONS

In conclusion, our data confirm the existence of more severe brain involvement in DM1 than in DM2, by both neuroimaging techniques and functional tests. Our neuropsychological battery revealed the presence of more marked executive dysfunction and visuo-spatial constructive functional defects, in DM1 compared to DM2. Patients with expanded [CTG]n>500 showed greater risk for abnormal MRI imaging, severe cognitive involvement and a worse neuromuscular picture. The results of our study suggest that MRI can be considered a reliable tool for detecting and quantifying focal and/or diffuse WMHLs in DMs; furthermore, MRI seems to be specific in the case of polar-temporal WMHLs in DM1. SPECT results appeared less specific and less comparable to the other parameters considered.

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