A non-DM1, non-DM2 multisystem myotonic disorder with frontotemporal dementia: phenotype and suggestive mapping of the DM3 locus to chromosome 15q21-24

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Summary

The majority of proximal myotonic myopathy syndromes reported so far have been related to the myotonic dystrophy (DM) type 2 (DM2) mutation, an expanded (CCTG)n repeat in the ZNF9 gene. Here, we describe the phenotype and the histological features in muscle and brain of the first large pedigree with a non-myotonic dystrophy type 1 (DM1) non-DM2 multisystem myotonic disorder associated with severe frontotemporal dementia. Thirty individuals from three generations underwent detailed neurological, neuropsychological, electrophysiological, brain imaging and molecular analyses. Ten of them had proximal muscle weakness at onset, clinical/electrical myotonia and DM-type cataracts. The mean age at onset was 46.7 ± 12.6 years (range: 32–69). Dementia was observed later in the course of the disease. On muscle biopsies, rare nuclear clumps, rimmed vacuoles and small angulated type 1 and type 2 fibres were seen early in the disease. They were replaced by fibrous adipose tissue at later stages. Immunohistochemical analysis of myosin heavy chain isoforms showed no selective fibre type atrophy—both type 1 and type 2 fibres being affected. Cortical atrophy without white matter lesions was seen on brain MRI. A brain single photon emission computed tomography (SPECT) study revealed marked frontotemporal hypoperfusion. Post-mortem examination of the brains of two patients showing prominent frontotemporal spongiosis, neuronal loss and rare neuronal and glial tau inclusions suggested frontotemporal dementia. Western blot analyses of the tau protein showed a triplet of isoforms (60, 64 and 69 kDa) in neocortical areas, and a doublet (64 and 69 kDa) in subcortical areas that distinguish our myotonic disorder from other’s myotonic dystrophies. Molecular analyses failed to detect a repeat expansion in the DMPK and ZNF9 genes excluding both DM1 and DM2, whereas a genome-wide linkage analysis strongly suggested a linkage to chromosome 15q21-24. This previously unreported multisystem myotonic disorder including findings resembling DM1, DM2 and frontotemporal dementia provides further evidence of the clinical and genetic heterogeneity of the myotonic dystrophies. We propose to designate this disease myotonic dystrophy type 3, DM3.

Keywords: myotonic dystrophy; DM1; PROMM/DM2; DM3; frontotemporal dementia
Introduction

Myotonic dystrophies (DM) are a genetically heterogeneous group of diseases. At least two different genetic entities are known: myotonic dystrophy type 1 (DM1) and type 2 (DM2). Myotonic dystrophies are clinically characterized by progressive myopathy, myotonia, posterior subcapsular cataracts, and multisystem involvement. They are transmitted with autosomal dominant inheritance. The mutation responsible for DM1 is a (CTG)n repeat expansion in the 3'-untranslated region of the DM protein kinase gene (DMPK) on chromosome 19q13.3 (Brook et al., 1992). The second form of myotonic dystrophy, DM2, is caused by a large (CCTG)n repeat expansion in intron 1 of the ZNF9 gene on chromosome 3q21 (Ranum et al., 1998; Day et al., 1999; Liquori et al., 2001). The DM1 and DM2 genes are transcribed into aberrant RNAs, with expanded CCUG and CCUG, respectively, which aggregate in nuclear foci (Taneja et al., 1995; Davis et al., 1997; Mankodi et al., 2003) and sequester RNA-binding protein of the muscleblind family (Mankodi et al., 2001; Fardaei et al., 2002). Recent studies in DM1 and DM2 point to abnormal regulation of the alternative splicing of various transcripts including insulin receptor, CIC-1 chloride channel, myotubularin, troponin Ic and microtubule-associated protein (MAP) tau (Philips et al., 1998; Savkur et al., 2001; Sergeant et al., 2001; Buj-Bello et al., 2002; Charlet et al., 2002). This common underlying mechanism of a toxic gain of function by the expanded RNAs might explain the phenotypic similarities between DM1 and DM2.

The majority of families studied with proximal myotonic myopathy syndrome (PROMM) and a single family with a proximal myotonic dystrophy characterized by a more severe muscular phenotype (Udd et al., 1997) have expanded (CCTG)n repeats in ZNF9 (Ricker et al., 1999; Day et al., 2003; Udd et al., 2003), resulting from an ancestral founder event (Bachinski et al., 2003; Liquori et al., 2003). Here, we describe a novel multisystem myotonic disorder unlinked to the DM1 and DM2 loci, associated with severe frontotemporal dementia in a large French pedigree. We report the results of detailed clinical, neuropsychological, brain imaging and histological studies of muscle and brain, as well as DNA analyses including the result of a genome-wide linkage analysis. This original disorder expands the clinical and genetic spectrum of multisystem myotonic diseases.

Patients and methods

Clinical assessment and diagnostic criteria

The genetic study was approved by The ethics committee of The CCPRB Pitie–Salpe`trie`re and CCPRB Paris–Necker. Thirty individuals underwent muscle strength evaluations with the modified Medical Research Council scale. Action or percussion myotonia was evaluated at room temperature in proximal and distal muscles and, after warming and cooling, in thenar eminence muscles. All individuals except one (Patient II-4) received four limb EMG including detection and recordings of motor and sensory conduction velocities. All individuals were screened according to their Mini-Mental State Examination score (Folstein et al., 1975). Neuropsychological evaluations were proposed to individuals with a score <26, indicative of cognitive dysfunction. All individuals underwent ophthalmologic slit lamp examinations and laboratory tests. Family members were considered to be affected when at least one of the following symptoms was present: early-onset dementia (before age 70), bilateral cataracts before the age of 50, myopathy or electrical myotonia. Dementia was diagnosed according to DSM-IV criteria (American Psychiatric Association, 1994).

Neuropsychological tests

Eight patients (II-4, II-5, II-6, II-8, III-9, III-31, III-36, III-37) were tested with a neuropsychological battery including the Wechsler Adult Intelligence Scale-Revised (Wechsler, 1989), the Mattis Dementia Rating Scale (Schmidt et al., 1994), the Wechsler Memory Scale (Wechsler, 1974), the Rey–Osterrieth complex figure copy (Rey, 1970), two 2-minute verbal fluency tasks (words with P as first letter and animals) (Cardebat et al., 1990) and the oral confrontation naming of 80 pictures (Deloche and Hannequin, 1997). Some patients were not able to perform all subtests, in particular due to severe visual disability caused by cataracts. Two patients were tested twice to evaluate cognitive deterioration with time (II-6, III-36).

Brain imaging

All affected individuals except for Patient IV-1 underwent brain MRI (II-4, II-6, II-7, II-8, III-9, III-31, III-36, III-37) or CT scan (II-5). On six patients (II-4, II-6, III-9, III-31, III-36, III-37), brain perfusion was evaluated with Tc-99 m ECD (Tc-99 m technetium ethyl cysteinate dimer single-photon emission computed tomography (SPECT).

Muscle histology

Quadriiceps muscle biopsies were performed in four patients (II-5, II-7, III-36, III-37) and post-mortem muscle samples were obtained from patients II-6 (quadriiceps) and II-8 (deltoid). Cryosections were stained with haematoxylin and eosin (H&E), Periodic Acid-Schiff (PAS), Sudan black and red, and Gomori’s trichrome. The following enzymatic activities were revealed by histochemical or histoenzymological methods: NADH-tetrazolium reductase, succinate dehydrogenase, menadione-linked (α-glycerophosphate dehydrogenase) and ATPase (pH 4.3, 4.6 and 9.4). Sections were immunostained with antibodies obtained from Novocastra Laboratories (Le Perray en yvette, France) against: spectrin (diluted 1:50); desmin (1:100); dystrophin 1 (1:2), 2 (1:4) and 3 (1:2); merosin (1:1000); α-sarcoglycan.
(1:100); δ-sarcoglycan (1:50); γ-sarcoglycan (1:100); and from Sigma Chemical Co Laboratories, St Louis, MO, USA against fast myosin heavy chain (MHC) (1:40); and slow MHC (1:80) (Sigma Chemical Co Laboratories). Sural nerve samples were processed by routine methods for optical and ultrastructural examination (Patients II-6 and II-8).

Other investigations
Cardiac function (standard ECG, 24-hECG, echocardiography) was investigated in all patients. Three affected patients had MRI or CT scans of skeletal muscles (II-6, II-7, III-36).

Creatine kinase, lactate, pyruvate, lactate dehydrogenase, aldolase, fasting glucose, thyroid stimulating hormone and alkaline phosphatases levels were measured in all individuals. In addition, two patients (II-6, III-36) had 5-h oral glucose tolerance tests including quantification of insulin levels.

Autopsy findings and neuropathological study
Post-mortem examination procedures
An autopsy was carried out on Patients II-6 and II-8. All intrathoracic, intraabdominal, retroperitoneal viscera, brain, spinal cord and muscles samples (quadriceps in Patient II-6, and deltoid in Patient II-8) were examined.

The left cerebral hemisphere was fixed in formalin. Paraffin-embedded coronal sections (7 mm) were stained by Haematoxylin and Eosin (H&E), Bodian-Luxol and modified Bielschowsky silver impregnation methods, and immunostained with antibodies obtained from DAKOPATTS, Trappes, France against β-amyloid protein (diluted 1:100), ubiquitin (1:200), glial fibrillary acidic protein (GFAP; monoclonal anti-GFAP, 1:300), prion protein (PrP) (1:50) and human tau protein (1:1000). Immunostaining was performed with antibodies against tau that are phospho-independent (MG19, AD190, AD192), phospho-dependent (AD2, AD294, AD199) and against a pathological site (Ser422P) (Buée et al., 2000).

Biochemical studies of tau protein
Pathological tau proteins were quantified by western blots on total brain tissue homogenates (Patients II-6 and II-8) as previously described (Delacourte et al., 1999).

Molecular analyses
Genomic DNA was extracted from peripheral blood leukocytes in 30 individuals, including nine affected individuals (II-4, II-5, II-6, II-8, III-9, III-31, III-36, III-37, IV-1) after informed consent was obtained. The DNA was not available for Patient II-7. For clarity, only a reduced pedigree is shown in Fig. 1 and not that for all 30 individuals.

The (CTG)n repeat sizes in the DMPK gene were determined in three patients (II-5, II-8, III-31) by two Southern blot analyses using EcoRI/B2.7 and Pol/UM10M6 (with permission of P. Harper), and by PCR with primers DM101/102 (Brook et al., 1992) flanking the trinucleotide expansion region. The repeat motif, (TG)n (TCTG)n (CCTG)n, located in the first intron of the ZNF9 gene (Liquori et al., 2001) and expanded in DM2, was PCR-amplified in eight patients (II-5, II-6, II-8, III-9, III-31, III-36, III-37, IV-1) using the 6 FAM™-labelled forward primer 5'-GGACTTGGAAATTGAGTGAATTCTGGCC-3' and the reward primer 5'-AATCATTACGACGTCACTCCAGGC-3'. Normal allele length varies from 188 to 242 bp in a control population of 30 normal individuals.

Non-DM1, non-DM2 multisystem myotonic disease
The genes encoding for amyloid precursor protein (exon 16 and 17), presenilin 1 and MAP-tau were studied by sequence analysis in patients II-6 and II-8, using previously described methods (Campion et al., 1995, 1999; Dumanchin et al., 1998). Karyotyping was performed in four patients (II-5, II-6, III-36, IV-1).

Genome-wide screen
The Linkage Marker Set MD 10 (Applied Biosystems, Foster City, CA, USA) formed the core marker set for the genome-wide screen. These 400 microsatellite markers, labelled with fluorescent dyes (FAM™, HEX™, NED™), are distributed at an average marker density of 10 centimorgans (cM); roughly every 10 million bases in the genome, and have an average heterozygosity of 75%. Centre National de Génotypage (CNG) has developed a protocol for robust co-amplification of up to six of these markers in a single reaction using dual 384-well GeneAmp® PCR 9700 cyclers (Applied Biosystems) and an automated procedure for PCR and purification set-up. All PCR reaction mixtures were prepared with a 96-tip head Automation Partnership BasePlate liquid handling robot (Matrix Apogent, Wilmslow, Cheshire, UK). PCR was carried out with 4 μL of DNA (diluted to 5 ng/μL) + 6 μL of PCR mix. The PCR fragments obtained were pooled and purified before separation on automatic sequencers. The purified dye-labelled fragments were separated according to size on MegaBACE 1000 96-capillary sequencers (Amersham Biosciences, Saclay, France). After injection (45s at 3 kV), the samples were run for 65 min at 10 kV using data collection software (Instrument Control Manager, version 2.1).

After collection, raw data from runs were transferred to an independent team for blind analysis. First, automatic genotyping was performed using Genetic Profiler software (version 1.1, Amersham Biosciences) applied to the raw MegaBACE data (i.e. trace processing, fragment sizing, allele calling, and assignment of genotype quality scores). Fragments of a similar estimated size for the same markers in the 96 samples of a single run were clustered providing an allele assignment. A quality score was assigned to each genotype by measuring the deviation of the observation from the median value for the allele, weighted by the standard deviation of the distribution over all observations.

Before statistical analysis, rigorous genotype quality assurance was performed to ensure accurate binning of alleles. Consistency of the data with Mendelian inheritance and lack of recombination between loci was evaluated using Pedcheck (O’Connell and Weeks, 1998) and other appropriate software.

Fine mapping
Additional linkage analyses were performed using microsatellite markers spanning the DMI locus (D19S217, DM101/102, D19S412) and the DM2 locus (D3S1267, D3S1589, D3S3606, D3S1292, D3S3637, D3S1569). Other loci known to cause myotonia—the muscle sodium channel SCN4A on 17q, and the chloride channel gene CIC-I on 7q—were genotyped with markers spanning the SCN4A (D17S782, D17S944, D17S949) and CIC-I regions (D7S640, D7S684, D7S661, D7S636, D7S798).

Statistical linkage analysis
We performed parametric LOD score linkage analyses assuming an autosomal mode of inheritance of disease. The disease allele frequency was set to 0.001. Since the age dependent penetrance curve for the disease gene is unknown, a conservative approach
Fig. 1 Reduced pedigree and haplotype analysis of the 15q region. All individuals that were taken into account for the multipoint linkage analysis are shown on this pedigree. Filled symbols indicate the affected patients. For all other individuals, the status is unknown. Subscript numbers refer to current age or age at death; 0 means that death occurred before age 1. The affected haplotype is indicated by grey shading for individuals for whom the phase is known. For the individuals in whom the marker was uninformative, the allele included in the haplotype is indicated by stippling. The DNA was not available for Patient II-7 for whom the haplotype, which has been deduced, is noted in italics and bracketed.
was used and only the affected members were included in the analysis. All remaining relatives were treated as ‘unknown phenotype’ (Fig. 1). We assumed no phenocopies.

Genome-wide linkage analyses were carried out in the original pedigree by computing two-point LOD-scores using the MLINK program from the LINKAGE package (Lathrop et al., 1984). The size and the complexity of our original pedigree limited fine mapping because it was not possible to simultaneously use more than two markers. To perform multipoint linkage analyses, we had to ignore the loop and withdraw four sibships from the youngest generations (offspring of Patients II-8, II-10, and offspring issued from a second marriage for Patient II-6 and Patient III-31 that are not shown on Fig. 1). We postulated that a small amount of the linkage information would be left out as these sibships did not contain any affected member and since the non-founder parent had DNA available. The resulting ‘reduced’ pedigree had four generations (see Fig. 1), 22 subjects (I-2, I-3, II-4, II-5, II-6, II-7, II-8, II-9, II-10, II-11, II-12, II-14, II-16, III-9, III-31, III-34, III-36, III-37, III-38, III-40, III-41, IV-1), 10 affected (II-4, II-5, II-6, II-7, II-8, II-9, II-10, II-11, II-12, II-14, II-16, III-9, III-31, III-34, III-36, III-37, III-38, III-40, IV-1) and 17 with DNA (II-4, II-5, II-6, II-7, II-8, II-9, II-10, II-11, II-12, II-14, III-9, III-31, III-34, III-36, III-37, III-38, III-40, IV-1). DNA was not available for one affected individual (II-7).

Multipoint LOD score analyses were performed using the FASTLINK package (Cottingham et al., 1993). Marker allele frequencies were set to their maximum likelihood values estimated with the computer program ILINK from the LINKAGE package (Lathrop et al., 1984). Genetic distances between markers were estimated from the data and compared with the Marshfield genetic location database.

To evaluate the robustness of our parametric linkage analyses with respect to our genetic assumptions, we performed non-parametric linkage analyses with the Merlin program (Abecasis et al., 2002), which allows non-parametric linkage analyses of moderately-sized pedigrees. The Z-all statistic compares the observed identity-by-descent sharing among all affected family members to that expected under the null hypothesis of no linkage (Whittmore et al., 1994; Kruglyak et al., 1996). The Z-all statistic is assumed to asymptotically follow a standard normal distribution.

We evaluated the power of our pairwise genome scan linkage analysis through simulations using the SLINK package program (Weeks et al., 1990). Five hundred replicates of the reduced pedigree structure were simulated using our dominant genetic model, and under the hypothesis of tight linkage ($\theta = 0.01$) between a DM-susceptibility locus and a polymorphic marker (eight alleles, heterozygosity rate = 78%). Pairwise LOD linkage analyses were then conducted in each replicate to calculate the maximum LOD-score over the recombination fraction. The means of statistics as well as the power at different threshold values were derived. The power of the pairwise linkage analysis was given by the number of replicates out of 500 having a maximum LOD value greater than the threshold value.

### Results

Ten patients over three generations (Fig. 1) had a dominant disease characterized by proximal weakness, clinical and/or electrical myotonia, early-onset dementia and early-onset cataracts (II-4, II-5, II-6, II-7, II-8, III-9, III-31, III-36, III-37, IV-1) (Table 1). The mean age at onset was 46.7 ± 12.6 years (range: 32–69), and was markedly earlier in the third (35.5 ± 3.3 years) than in the second generation (55.6 ± 9.3 years). Patient IV-1 was not considered for age at onset as he was clinically asymptomatic and was found to be affected by EMG.

The first symptom was proximal muscular weakness in six patients (II-6, II-8, III-9, III-31, III-36, III-37), cataracts in case II-7 and dementia in two cases (II-4, II-5). Four patients died between age 61 and 69 years (mean age: 64.3 years) and their mean disease duration was 11.5 ± 3.6 years (range: 7–16 years). The cause of death was pneumonia secondary to swallowing difficulty in three cases and respiratory failure in one case. Patient III-9 was not considered in this calculation because the cause of death was suicide at age 48 years. The individuals of the first generation were dead, but the male I-2 had dementia and died at age 56 in a psychiatric hospital. His sister (I-1) was also demented when she died at 54.

The risk of the disease is sex and age-dependent. Although not apparent on the reduced pedigree shown on Fig. 1, we should mention a sex-ratio disequilibrium in affected individuals (one male for nine females), and in offspring of Patients I-2/I-3, II-7 and II-8 (eight males for 21 females). A number of children died before the age of 1 year of various aetiologies that were not apparently related to the disease (meningitis, cerebral atrophy, psychosis, gallstones). Three of them were in the fourth generation. Two died at 3 years of age (II-14, III-38), one at 2 years (III-34), and one at 1 year (III-40). They had idiopathic death by suicide, and one death by meningitis. One patient (II-16) died at age 13 years because the cause of death was suicide at age 48 years. The other cases died at age 44 years at a psychiatric hospital. Patient III-9 was not considered in this calculation because the cause of death was suicide at age 48 years. The individuals of the first generation were dead, but the male I-2 had dementia and died at age 56 in a psychiatric hospital. His sister (I-1) was also demented when she died at 54.

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### Table 1 Phenotype in ten affected patients

<table>
<thead>
<tr>
<th>Patients</th>
<th>II-4</th>
<th>II-5</th>
<th>II-6</th>
<th>II-7</th>
<th>II-8</th>
<th>III-9</th>
<th>III-31</th>
<th>III-36</th>
<th>III-37</th>
<th>IV-1</th>
</tr>
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<tbody>
<tr>
<td>Age at onset (years)</td>
<td>69</td>
<td>58</td>
<td>57</td>
<td>49</td>
<td>45</td>
<td>40</td>
<td>35</td>
<td>35</td>
<td>32</td>
<td>24*</td>
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<tr>
<td>Disease duration (years)</td>
<td>4</td>
<td>7</td>
<td>11</td>
<td>12</td>
<td>16</td>
<td>8</td>
<td>9</td>
<td>14</td>
<td>6</td>
<td>7</td>
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<tr>
<td>Dementia</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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<tr>
<td>Myotonia</td>
<td>na</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Cataracts</td>
<td>+70</td>
<td>+65</td>
<td>+63S</td>
<td>+94S</td>
<td>+59</td>
<td>+47</td>
<td>–</td>
<td>+40</td>
<td>+36</td>
<td>–</td>
</tr>
<tr>
<td>Other signs</td>
<td>D</td>
<td>D</td>
<td>IR</td>
<td>–</td>
<td>–</td>
<td>Gallstones</td>
<td>Psychosis</td>
<td>Gallstones</td>
<td>MR</td>
<td>DG</td>
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<tr>
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<td>***</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>na</td>
</tr>
<tr>
<td>Frontal hypoperfusion</td>
<td>****</td>
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<td>+</td>
<td>+</td>
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</table>
| *Asymptomatic; **Cognitive deficits but not all criteria for definitive dementia; ***on brain MRI; ****on SPECT evaluation; $\dagger$patient deceased; $\ddagger$death by suicide. + = present; – = absent; $\S$cataracts needing extraction; $\dagger$patient deceased; $\ddagger$death by suicide; C = clinical myotonia; D = diabetes; DG = diabetes gravis; E = electrical myotonia; IR = insulin resistance; MR = mental retardation; na = not available.

Age at onset is given for weakness and dementia, age at detection or at last examination is given for cataracts.
dehydration, traumatism . . .) or of unknown causes. However, none of the children for whom the cause of death is known had findings suggestive of a congenital and more severe form of the disease.

**Phenotype**

Eight patients (II-4, II-6, II-7, II-8, III-9, III-31, III-36, III-37) developed proximal muscle weakness between age 32 and 69 years. The muscle deficit initially involved proximal and axial musculature. Facial musculature was spared. Three patients (III-31, III-36, III-37) mentioned intermittent muscle pain. The clinical course led progressively to major disability with severe proximal weakness and atrophy and, later on, distal weakness, atrophy and neck extensor weakness responsible for drooped head (Fig. 2). The mean disease duration before the patients became wheelchair-bound was 7.4 ± 4.3 years. Grip or percussion myotonia was elicited in three patients. The myotonia was variable and difficult to detect clinically or by EMG. It was mainly noted in hand, deltoids and thigh muscles.

Low amplitude and polyphasic motor unit potentials, consistent with a myopathic pattern, were recorded by EMG in proximal muscles in Patients II-6, II-7, III-9, III-36, III-37. In six patients (II-6, II-8, III-9, III-31, III-37, IV-1), spontaneous discharges were recorded. The action potential frequencies (50–100 Hz), the brief duration of the discharges (500 ms to 5 s, and rarely as long as 10 s) and the typical decrease in frequency and amplitude were characteristic of myotonic discharges. As in DM2, the myotonic runs were difficult to detect. They could not be evidenced in three out of the 10 patients who were examined. In addition, myotonic runs were variable in time: they were not recorded on each examination in patients who underwent several successive EMG recordings. No effect of warming or cooling could be detected. Motor and sensory conduction velocities were normal in all patients. Creatine kinase levels were normal to twice the normal upper limit. MRI or CT scans of muscles showed moderate atrophic changes in paraspinal muscles in Patient II-6 and marked fatty degeneration in paraspinal muscles, vastus lateralis and muscles of the posterior compartment of the thighs in Patients II-7 and III-36.

Six patients (II-4, II-5, II-6, II-7, II-8, III-36) had early-onset dementia, and one (III-9) had cognitive troubles but without all the criteria for definite dementia (Table 2). The mean age at onset was 56.8 ± 10.3 years (range: 39–69 years). Dementia was initially characterized by a prominent memory deficit, constructional impairment and word-finding problems with a progressive reduction of speech in two patients (II-8, III-36). The mean duration before mutism was 4.0 ± 1.0 years. In other patients (II-4, II-5, II-7, III-36), behavioural modifications with verbal disinhibition, agitation, aggressiveness and apathy, were the predominant signs at onset. The cognitive deterioration fulfilled the criteria for frontotemporal dementia (Lund and Manchester Groups, 1994; Neary et al., 1998). In Patient II-8, the clinical course was characterized by seizures and myoclonic jerks. Patient III-36 was mentally retarded. However, her cognitive condition deteriorated rapidly at age 39 years. She developed apathy, emotional lability, severe loss of speech and a comprehension deficit, and used coarse language and became aggressive. The rapid deterioration and the SPECT follow-up showing severe progressive decrease of frontal perfusion were consistent with the diagnosis of dementia (Fig. 3). Furthermore, it is remarkable that two patients (III-36, III-37) were mentally retarded and that Patient III-9 developed a schizophrenic-like psychosis at age 35 years. She was admitted to a psychiatric hospital after several episodes of severe depression, suicide attempts and paranoid delusions with auditory hallucinations.

Bilateral cataracts were found in eight patients (II-4, II-5, II-6, II-7, II-8, III-9, III-36, III-37) and needed extraction in two patients (II-6, II-7). They were indistinguishable from cataracts found in DM patients with typical iridescent and posterior subcapsular lens opacities (Table 1). The mean age at onset was 52.0 ± 12.6 years (range: 36–70 years).

Endocrine disturbances were limited to diabetes mellitus (Patients II-4, II-5), glucose intolerance with peripheral insulin resistance (Patient II-6) and diabetes gravis (Patient III-37). Other biological parameters, including lactate and pyruvate, were normal. Gallstones were noted in four patients (II-5, II-6, II-8, III-31). No patient had frontal baldness, hearing loss or sleep disturbances. Cardiac function was normal in all patients.

**Brain imaging**

Brain MRI or CT-scans revealed mild to severe cortical atrophy with prominent enlargement of Sylvian fissures in Patients II-4, II-5, II-6, II-7 and II-8. None of the patients had white matter abnormalities on brain MRI. A marked decrease in frontal lobe perfusion was observed in all patients who underwent SPECT scans (Fig. 3).
Muscle histology

In the initial stage of the disease, muscle biopsies showed moderate histopathological changes including a few central nuclei, rare nuclear clumps and scattered atrophic fibres of both types (Fig. 4A, B, C). Some specimens (III-37) had occasional rimmed vacuoles (Fig. 4C). No fibre type grouping (Fig. 4D) or targetoid fibres were observed. There were no histological hallmarks suggestive of DM1 (ring fibres, subsarcolemmal masses, nuclear chains, type 1 fibre atrophy) or DM2 (preferential type 2 fibre atrophy, type 2 nuclear clumps fibres), and no mitochondrial alterations (ragged-red fibres, abnormal succinate dehydrogenase activity). These early changes, although non-specific, were consistent with a primary muscle disorder. The muscle biopsy was normal in only one patient (III-36). Late in the disease (II-5, II-6, II-7, II-8), extensive myofibre loss and fatty infiltration with moderate fibrosis were encountered. The histological pattern was heterogeneous, with grouped atrophic angulated fibres in some bundles (Fig. 4E and F), suggesting a co-existing denervation.

Immunohistochemical analyses of MHC isoforms revealed a few scattered atrophic fibres of both types at early stage and the absence of very atrophic fibres containing nuclear clumps (Fig. 4G and H). Similarly, no selective type 1 or type 2 atrophy could be detected in the severely affected muscle samples. However, both fibre types displayed distinct intensity of atrophic process, as atrophic type 1 fibres were mainly thin, irregular and scattered, whereas fast MHC immunolabelling detected very small type 2 fibres (less than 10 μm in diameter) with nuclear clumps (Fig. 4I and J). A small proportion of fibres—mainly normal-sized—expressed both fast and slow MHC isoforms, and were suggestive of regenerative fibres. Fibre type grouping was not observed. Immunolabelling for spectrin, dystrophin, merosin and sarcoglycans was normal and there was no abnormal desmin storage. Post-mortem optical and ultrastructural examinations of the sural nerve were normal in two patients (II-6, II-8).

Fig. 3 Brain SPECT study showing severe frontal hypoperfusion in Patient III-36: transverse (left) and sagital (right) sections.

| Table 2 Neuropsychological evaluation in eight patients |
|---------------------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Patients                        | II-4 | II-5 | II-6 | II-6 | II-8 | II-9 | III-31 | III-36 | III-36 | III-37 |
| Educational (years)             | 7    | 6    | 7    | 6    | 7    | 7    | (d)    | (d)    |       |       |
| Age at evaluation (years)       | 73   | 60   | 66   | 67   | 58   | 47   | 49     | 35     | 40    | 36     |
| Disease duration (years)        | 4    | 2    | 9    | 10   | 13   | 7    | 14     | 1      | 5     | 4      |
| MMSE                            | 22   | 17   | 19   | 18   | 17   | 22   | 27     | 14     | 15    | 24     |
| WAIS R-IQ                       | --   | **   | ***  | **   | --   | **   | ***    | ***    | ***   | **     |
| MDRS                            | --   | --   | $**  | $#   | ***  | *    | n      | --     | ***   | n      |
| WMS-MQ                          | --   | --   | *    | *    | ***  | n    | n      | n      | n     | *      |
| WMS                             | --   | --   | *    | *    | ***  | n    | n      | n      | n     | *      |
| Orientation                     | n    | ***  | n    | n    | ***  | n    | n      | n      | n     | *      |
| Mental control                  | n    | n    | n    | n    | **   | n    | **     | ***    | ***   | **     |
| Logical memory                  | *    | --   | *    | *    | **   | n    | *      | *      | **    | **     |
| Digit span                      | ***  | ***  | *    | ***  | ***  | n    | *      | **     | ***   | **     |
| Visual reproduction             | --   | --   | ND   | *    | *    | n    | n      | **     | **    | **     |
| Associative learning            | *    | --   | n    | ND   | ***  | n    | n      | *      | *     | n      |
| Rey’s figure copy               | --   | ***  | ND   | ND   | ***  | ***  | ***    | ***    | ***   | ***    |
| Verbal fluency                  | --   | ***  | ND   | ND   | ***  | ***  | ***    | ***    | ***   | ***    |
| Letter P                        | ***  | --   | *    | **   | **   | *    | n      | --     | ***   | *      |
| Animals                         | ***  | ***  | **   | ***  | ***  | n    | *      | --     | ***   | *      |
| Naming                          | ***  | ***  | ND   | ND   | ***  | n    | n      | --     | ***   | n      |

*Results ± 1 SD, **± 2 SD, ***± 3 SD below the age and sex-related means from the normative data. = not performed; $ = only verbal items (96) of the MDRS (144) were used because of visual disability; (d) = developmental cognitive impairment; IQ = intelligence quotient, was the WAIS-R full scale IQ score for all patients except II-5 and II-6 for whom only the verbal IQ was available; MDRS = Mattis Dementia Rating Scale; MMSE = Mini-Mental State Examination; MQ = Memory Quotient, evaluated with the WMS; n = normal value; ND = not done because of visual disability; WAIS-R = revised Wechsler Adult Intelligence Scale; WMS = Wechsler Memory Scale.

Non-DM1, non-DM2 multisystem myotonic disease 1985
Neuropathology and tau protein isoforms

Patient II-6

On macroscopic examination, the brain was diffusely atrophic (975 g). On coronal sections, the caudate nuclei were flattened. Substantia nigra and locus coeruleus were normally pigmented. Histological examination demonstrated neuronal loss and microspongiosis in layers II and III in frontal (Fig. 5), temporal and insular cortices. The superior and middle frontal gyri were markedly affected. Cingulate, occipital cortices, hippocampus and the nucleus basalis of Meynert were preserved. Caudate nuclei showed neuronal loss, vacuolation and astrocytic gliosis (Fig. 5). No lesions were observed in the putamen, globus pallidus and thalamus. Pick bodies, as well as ballooned cells and Lewy bodies were absent. The white matter was normal. The cerebellum was normal, except for a mild white matter gliosis. Examination of the spinal cord revealed loss of anterior horn neurons, particularly in the lumbar and thoracic spinal cord. Rare chromatolytic neurons were found in the thoracic spinal cord. GFAP immunohistochemistry evidenced astrocytic gliosis in the frontal, temporal and parahippocampic cortex. Positive GFAP cells were aggregated in foci, with a laminar pattern, close to the spongiform changes (layers II and III). Immunohistochemical PrP analysis was negative. Immunostaining for ubiquitin was negative. Beta-amyloid antibodies did not detect amyloid angiopathy. Immunohistochemical analysis using phospho-independent tau antibodies were negative. Immunostaining with phospho-dependent tau antibodies found only scarce immunoreactive round neuronal inclusions in the temporal pole, insular cortex, brainstem, hypothalamus and caudate nucleus. Inclusions were more abundant in the nucleus basalis of Meynert. Few glial inclusions were evidenced in the putamen, the caudate nucleus, the frontal cortex and the inferior olivary complex. In the hippocampus, the entorhinal cortex and the temporal pole, a few neurofibrillary tangles and senile plaques were observed, consistent with normal ageing. Spinal cord, cerebellum and medulla displayed no inclusions.

Patient II-8

Macroscopic examination of the brain revealed bilateral diffuse cerebral atrophy (863 g). The substantia nigra and locus coeruleus were depigmented. The caudate nuclei were markedly atrophic. Histopathological findings were similar, but more severe than in Patient II-6. The major finding consisted of conspicuous micro and macrovacuolar spongiform degeneration of the cortical layers II and III, affecting prominently frontal, temporal, insular cortices and, less severely, parietal
The occipital cortex was spared. Considerable neuronal loss and reactive astrocytic gliosis were observed in the frontal and temporal cortices, particularly in the inferior frontal, superior temporal, parahippocampal gyri and hippocampus. Ammon’s horn was relatively spared. The caudate nuclei were also severely affected by spongiosis, neuronal loss and gliosis. The thalamus was less severely affected. The globus pallidus and the putamen were normal. Immunostaining with antibodies raised against β-amyloid protein, ubiquitin, GFAP and PrP were negative. As in Patient II-6, amyloid deposits, ubiquinated inclusions, Pick bodies, ballooned cells and Lewy bodies were absent, and the few neurofibrillary tangles detected in the hippocampus and entorhinal cortex were consistent with normal ageing. The cerebellum was normal. In the brainstem, neuronal loss and depletion of pigmented neurons in the substantia nigra and the locus coeruleus were observed. Neuronal loss was noted in the hypoglossal and olivary nuclei. Examination of the cervical spinal cord revealed a loss of anterior horn cells and few swollen chromatolytic neurons. Thoracic and lumbar spinal cord was not examined. The pattern of phospho-dependent immunostaining of the tau protein was similar to that of Patient II-6, although less prominent.

**Biochemical studies of tau isoforms**

Pathological tau proteins were observed only in Patient II-6. These proteins, consisting of 60, 64 and 69 kDa electrophoretic bands, were immunodetected in many neocortical areas, and especially in Brodmann areas 39, 38, 20 and 10. The occipital cortex was spared. The tau triplet had a class I profile, as in Alzheimer’s disease, but the signal was much weaker (1–2%) (Delacourte and Buée, 2000). Additionally, pathological tau proteins were detected in subcortical areas, such as the caudate nucleus or the striatum, with a distinct electrophoretic profile, mainly consisting of 64 and 69 kDa electrophoretic bands, as in progressive supranuclear palsy (class II tau pathology) (Buée and Delacourte, 1999; Delacourte and Buée, 2000). No tau pathology was detected in the cerebellum, muscle or liver samples. Native tau protein levels in neocortical and subcortical areas were similar to those found in controls.

**Molecular analyses**

Southern blots with B2.7 and M10M6 probes failed to detect amplification of the CTG repeat in the DMPK gene. The amplification of the repeat motif (TG)n (TCTG)n (CCTG)n in the first intron of the ZNF9 gene revealed heterozygosity in eight
patients (II-5, II-6, II-8, III-9, III-31, III-36, III-37, IV-1). The two alleles were in the normal range (204 to 242 bp), excluding a (CCTG)n repeat expansion. There were no mutations in the β-amylod precursor protein (exons 16 and 17), presenilin 1 and MAP-tau genes, and karyotypes were normal.

**Linkage analysis**

Our simulation study showed that a genome-wide scan analysis in our pedigree had good power (74%) to obtain LOD scores >1.0. With the pedigree structure and genetic model used in the study, and with the assumption of tight linkage (θ = 0.01) between the DM-susceptibility gene and a polymorphic marker, the expected maximum LOD-score value was 1.54, with a maximum possible value of 2.38. Only 11 markers (within nine chromosomal regions) gave LOD score values >1: 1q44.1 (D1S2836, LOD = 1.06); 5q44.1 (D5S428, LOD = 1.67); 8p23.3 (D8S264, LOD = 1.40); 9q22.32 (D9S287, LOD = 1.49); 15q11.13 (D15S1002, LOD = 1.30 and D15S165, LOD = 1.21); 15q22.24 (D15S153 and D15S131, LOD = 2.23); 19q13.33 (D19S902, LOD = 1.21); 22q11.22 (D22S393, LOD = 1.05); and Xp22.33 (DXS1060, LOD = 1.21).

Since the *DMPK* locus lies at 19q13, three additional markers (D19S217, DM101/102, D19S412) spanning the locus were genotyped. Reconstruction of the haplotypes excluded the *DM1* locus. Linkage to markers in the 3q21 (DM2) region was also rejected in our pedigree (D3S1292, LOD = −3.31), nor did we find LOD scores suggestive of linkage to other loci known to cause myotonia, the muscle sodium channel *SCN4A* on 17q (D7S944, LOD = −0.21) and the CIC-I chloride channel gene on 7q (D7S665, LOD = −3.61).

The highest LOD score value was obtained with marker D15S153 in the 15q21-24 area (Table 3), which was therefore selected for further fine mapping studies. We genotyped 13 polymorphic markers in the 63-cM interval bounded by loci D15S128 and D15S205. Most of these markers yielded positive LOD scores in pairwise analysis (Table 3). Again, the highest maximum LOD-score value was found within 15q21-24 (LOD = 2.23, with D15S970, D15S993). Similar parametric LOD-score results were obtained in the reduced pedigree. These results were robust with respect to our genetic model, as shown by the pairwise non-parametric linkage analyses (Table 3).

Multipoint linkage analysis yielded a maximum LOD score of 2.38 in the 10-cM interval between D15S970 and D15S114 (Fig. 6). Similarly, non-parametric linkage analysis of allelesharing among affected members gave a non-parametric Z-all score of 12.82 (P < 10−5), which is significant at the genome-wide level.

For D15S983 marker, the genotyping success rate was low due to poor DNA amplification and to difficult analysis of allelic profiles. Therefore, the results from nine individuals were rejected. As shown in Fig. 1, all the genotypes for individuals in the offspring of II-7/II-14 (III-31, III-34, III-36, III-37, III-38, III-40) were not validated for marker D15S983, therefore making it poorly informative. In multipoint analyses, as the density of markers is high, the decrease of the LOD value at this point is moderate (Fig. 6).

Haplotypes were established by minimizing the number of recombination events for 24 markers on chromosome 15q with the Merlin program (Abecasis et al., 2002), which finds the best set of inheritance vectors in the pedigree. All ten affected patients shared a ~10 cM haplotype, defined by recombination events in three affected relatives (Patients II-4, III-31 and III-37) that map the critical region between D15S970 and D15S114 (Fig. 1). The haplotypes were deduced for Patient II-7 (Fig. 1) because DNA was not available for this affected individual.

**Discussion**

The distinctive clinical features of the new disease entity described here are proximal weakness, clinical myotonia, early bilateral DM-type cataracts and early-onset dementia. The disease evolved in two stages. At onset, the patients had slowly progressive proximal muscle weakness, muscle pain, clinical/electrical myotonia, DM-type cataracts, mild creatine kinase elevation and non-specific myopathic histological features. Later on, the disease was characterized by severe weakness and atrophy in proximal, distal and neck extensor muscles, and severe frontotemporal dementia.

The risk of the disease is age-dependant, as shown by the great variability of age at onset (32–69 years) in affected patients. In addition, two individuals (Patient II-9 aged 58 years and Patient II-10 aged 63 years), who share the morbid haplotype are not clinically affected (see Fig. 1). The observed pattern of the disease distribution in the pedigree could suggest an X-linked mode of inheritance of DM. However, our genome scan results provided only weak evidence for an X-linked susceptibility locus (LOD = 1.21). Higher LOD scores were obtained in other autosomal regions. The nominal *P* value associated to this LOD score is 1%. We have estimated the empirical distribution of the LOD-score test in our chromosome X data, and found an estimated empirical *P* = 1.4%, in agreement with the theoretical expectations. These results show that, when there is no linkage, the probability of these outcomes is not low, especially in a genome-wide search implying multiple tests. Thus, weakly positive LOD scores can be observed due to random fluctuation.

All mandatory criteria for a DM-like disease were present in the family: (i) proximal muscle weakness; (ii) clinical/electrical myotonia; (iii) DM1-type cataracts; (iv) autosomal dominant inheritance; and (v) absence of CTG repeat size in the *DMPK* gene (Moxley et al., 2002). Furthermore, peripheral insulin resistance and gallstones are additional common features of both DM1 and DM2. Here, the severity of late muscular atrophy with marked fatty replacement resembles the proximal myotonic dystrophy phenotype described in a single family (Udd et al., 1997). The mean age at onset is earlier in the third than in the second generation in this family, which may be suggestive of anticipation—commonly observed in diseases caused by repeat expansions, in particular DM1, but not
considered a regular finding in DM2 (Day et al., 2003; Udd et al., 2003).

As in PROMM/DM2, electrical myotonia was variable and difficult to detect, and needed a detailed examination (Moxley et al., 2002). It is possible, therefore, that electrical myotonia was missed in three patients (II-5, II-7, III-36). In the early stage, muscle biopsies showed non-specific myopathic features, including atrophic type 1 and type 2 fibres, rare nuclear clumps and rimmed vacuoles. Analyses of MHC isoforms confirmed that both type 1 and type 2 fibres were affected in mildly as well as in severely affected muscles. Interestingly, the absence of preferential type 1 fibre atrophy differs from DM1 (Harper, 2001), and there was no histoenzymologic evidence for the preferential type 2 atrophy recently reported in DM2 (Bassez et al., 2001; Vihola et al., 2003). This disease differs therefore from other myotonic dystrophies with respect to pathophysiological alterations of myofibres. At later stages of the disease, there were additional neuropathic changes that may have contributed to severe muscle wasting. However, there was no electrophysiological or histological evidence that the early muscle weakness was caused only by denervation.

Most interesting is the spectrum of neurological deficits observed in the patients, which included dementia, mental retardation and possibly schizophrenia. The neuropsychological profile, frontal SPECT hypoperfusion and neuropathological features were consistent with frontotemporal dementia. The co-segregation of dementia with muscular symptoms in the oldest patients and the absence of mutations in the major genes responsible for familial dementias make it unlikely

![Fig. 6](image-url)
that two distinct diseases were associated. Mental retardation is frequent in congenital forms of DM1, but rare in DM2 (Moxley et al., 2002; Day et al., 2003; Udd et al., 2003). Other cognitive changes are limited in both DM1 and DM2 to moderate executive dysfunction—associated with white matter lesions on MRI and decreased frontal and temporal blood flow (Meola et al., 1999, 2003; Udd et al., 2003). Rapid and severe deterioration is, however, unusual. The association of a DM-like disorder with severe frontotemporal dementia, as well as the absence of white matter abnormalities on brain MRI, distinguish this disease from both DM1 and DM2. Furthermore, the neurological spectrum observed in the present family differs from that of an atypical PROMM family with seizures, extrapyramidal symptoms, hypersonnia, stroke-like episodes and white matter abnormalities on MRI (Hund et al., 1997). As for mental retardation seen in two patients in our family (III-36, III-37), it could be a consequence of consanguinity (Fig. 1). It may also be a sign of the disease, in which case the disease could lead to both developmental and neurodegenerative pathology, as in DM1.

The neuropathological features of the disease include spongiosis, neuronal loss and gliosis—mainly in the frontal and temporal cortices and the basal ganglia—consistent with a frontotemporal dementia. The absence of amyloid deposits, neuritic plaques, Lewy bodies, Pick bodies or other ubiquitinated inclusions and the rarity of tau inclusions distinguish this dementia from Alzheimer’s disease, Lewy body disease, Pick’s disease and dementia with ITSNU (Inclusions Tau Synuclein Negative Ubiquitinated) (Kertesz et al., 2000). Neurofibrillary tangles were scarce, as were neuronal and glial tau inclusions. Some neuronal loss and a few swollen chromatolytic neurons were present in anterior horn cells and hypoglossal nucleus, but ubiquitinated inclusions in many subcortical nuclei (Sergeant et al., 2001). The same distribution was observed in one DM2 patient (Udd et al., 2003; C.A. Maurage and N. Sergeant, personal communication). The major molecular mechanism underlying tau pathology in DM1 and DM2 is an abnormal alternative splicing of tau mRNA, decreasing the level of isoforms containing exon 2 and 3, which results from a negative trans-dominant effect of CUG expansion on RNA maturation. The major tau isoform had an apparent molecular weight of 60 kDa, whereas 64 kDa and 69 kDa tau were present at lower and traces levels, respectively. This pattern of tau isoforms determines a rather specific tauopathy characteristic of DM type 1 and type 2 (class IV of tau pathology) (Vermersch et al., 1996; Sergeant et al., 2001; Seznec et al., 2001; Udd et al., 2003; C.A. Maurage and N. Sergeant, personal communication). In the family we describe here, neither the expression nor the splicing of the tau protein appeared to be altered. However, unusual pathological electrophoretic profiles were observed in one of the two patients, consisting of a class I triplet (60, 64 and 69 kDa) in neocortical areas, and a class II doublet (64, 69 kDa) in subcortical areas. Both profiles are completely different from those of DM1 and DM2 since the pathological tau aggregation includes isoforms with exons 2 and 3. This suggests that tau RNA maturation is most likely not the cause of neurodegeneration. The pathological mechanism underlying the brain pathology appears therefore to differ from that of other myotonic dystrophies.

We have conducted a genome-wide screen in this multi-generational pedigree, whose results are strongly suggestive for a candidate DM-susceptibility locus at 15q21-24 under a dominant model. Our maximum LOD-score value of 2.38 corresponds to a nominal $P < 10^{-3}$. When corrected for the multiple testing according to Lander and Kruglyak (1995), none of our LOD-scores reached the threshold of significance ($P < 2.2 \times 10^{-5}$), although in our multipoint non-parametric linkage analyses, the nominal $P$ value ($Z\text{-all} = 12.82$, $P < 10^{-5}$) exceeded this threshold. The Lander and Kruglyak guidelines have been criticized, however, because they do not take into account the power of the analysed samples, which is sufficient in our pedigree and with the model used to detect linkage at a nominal $P \geq 0.1\%$ (power $\geq 41\%$), but not at a lower nominal $P \leq 10^{-4}$ (power $\sim 0\%$). Our simulations also showed that a LOD-score value of 2.38 is the highest that can be obtained with our data and support linkage for a DM-susceptibility locus on chromosome 15q22-24. The linkage region remains relatively large (~10 cM), however, and could not be narrowed down in this pedigree. Additional linked families will be needed to refine the locus.

In conclusion, we have described a novel multisystem myotonic disorder including features resembling DM1, DM2 and a frontotemporal dementia, without repeat expansions in the $DMPK$ and $ZNF9$ genes. This previously unreported disease expands the clinical spectrum of myotonic dystrophies and increases their genetic heterogeneity. We propose that it be designated myotonic dystrophy type 3, DM3.

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