Centronuclear myopathy related to dynamin 2 mutations: Clinical, morphological, muscle imaging and genetic features of an Italian cohort

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Abstract

Mutations in dynamin 2 (DNM2) gene cause autosomal dominant centronuclear myopathy and occur in around 50% of patients with centronuclear myopathy. We report clinical, morphological, muscle imaging and genetic data of 10 unrelated Italian patients with centronuclear myopathy related to DNM2 mutations. Our results confirm the clinical heterogeneity of this disease, underlining some peculiar clinical features, such as severe pulmonary impairment and jaw contracture that should be considered in the clinical follow-up of these patients. Muscle MRI showed a distinct pattern of involvement, with predominant involvement of soleus and tibialis anterior in the lower leg muscles, followed by hamstring muscles and adductor magnus at thigh level and gluteus maximus. The detection of three novel DNM2 mutations and the first case of somatic mosaicism further expand the genetic spectrum of the disease. © 2013 Elsevier B.V. All rights reserved.

Keywords: DNM2; Centronuclear myopathy; Muscle MRI; ‘Necklace’ fibers; Somatic mosaicism

1. Introduction

Mutations in dynamin 2 (DNM2) gene cause autosomal dominant (AD) centronuclear myopathy (CNM) and occur in around 50% of patients with CNM [1]. DNM2-related CNMs are generally clinically milder than the X-linked neonatal forms associated with mutations in myotubularin gene (MTM1; MIM# 300415) [2], and the autosomal recessive (AR) forms related to mutations in the membrane-bending protein amphiphysin 2 (BIN1; MIM# 601248) [3].

A wide spectrum of severity has been described in DNM2-related CNM, ranging from neonatal onset with severe course to adult onset with a milder phenotype [4–7].

Facial weakness, bilateral ptosis and ophthalmoparesis are seen in most patients, variably associated with distal
muscle atrophy, finger and ankle contractures and pes cavus [8]. The three histopathological hallmarks on muscle biopsy in DNM2-related CNM are hypotrophy and predominance of type I fibers, radial arrangement of sarcoplasmic strands and centrally located nuclei [1]. To date, 19 different CNM-related DNM2 mutations have been reported in approximately 100 families [9]. Mutations affecting the middle domain (MD) are generally associated with a relatively mild clinical course [5,10], while those in the pleckstrin-homology (PH) and GTPase effector (GED) domains of the protein are more frequently found in more severe variants [4,9]. DNM2 mutations affecting the PH domain have also been associated with dominant intermediate Charcot–Marie–Tooth Disease (CMTDIB) [11], and in an axonal variant of the same group of disorders, CMT2M [12]. We report clinical, morphological, muscle imaging and genetic findings from 10 unrelated Italian patients affected by CNM caused by mutations in DNM. We also report three novel mutations and a somatic mosaicism.

2. Patients and methods

We systematically collected data regarding age and mode of onset, functional abilities, progression of the disease, muscle power and severity of contractures in 10 patients (4 males, 6 females) of Italian origin with CNM and confirmed DNM2 mutation. Serum creatine kinase (CK) levels, electromyography (EMG) and nerve conduction study (NCS), muscle biopsy, electrocardiogram (EKG), echocardiogram, pulmonary function tests were available in all patients. Diagnostic muscle biopsy was performed in all patients after written informed consent. Frozen cryosection were processed according to standard histological and histochemical techniques.

2.1. Muscle MRI

MRI studies were performed using 1.5-T MR scanners in different medical centres. MRI T1-weighted transverse images were obtained from trunk and the legs, selecting the axial plane with respect to the long axis of the body. The sections were 5 mm thick, and the gap between sections was 50 mm. All patients were fully cooperative, and no sedation or general anesthesia was required.

2.2. Sequencing analysis

Genomic DNA was extracted from peripheral blood in all patients using DNA Extraction Kit (Qiagen, Hilden, Germany). Family members of the probands were investigated for the presence of the mutation identified by direct sequencing where DNA was available. The genomic sequence of DNM2 was used to design PCR primer pairs to amplify all 22 coding exons belonging to the four major DNM2 isoforms as previously described [5]. Nucleotide sequence determination was performed by cycle sequencing using a BigDye Terminator DNA sequencing kit (Applied Biosystems, Hercules, CA). Results were compared to the human genomic DNM2 database sequences NM_001005360.1 and NM_001005360.2.

Polyphen-2 (http://genetics.bwh.harvard.edu/pph2/) and SIFT (http://sift.jcvi.org/) software were utilized to characterize the possible impact of the non synonymous amino acid substitutions identified.

2.3. SNaPshot analysis

To quantify the level of the c.1872dupAGCTGG mutation in the asymptomatic father of patient 6 with suspected somatic mosaicism, SNaPshot analysis was performed in blood of patient 6 and blood, cells from urinary tract, hair and saliva from his father. PCR was performed on complementary DNA with forward primer (5’-TAGAGCCATTCCCTCCTGGG-3’) combined with backward primer (5’-GAAAATGACCTGTCCTGGGGA-3’).

SNaPshot is a primer extension method that relies on the addition of a single dye-labeled dideoxy nucleotide (either wild-type or mutant nucleotide) to primers localized adjacent to the mutation under examination [13]. The primer sequence used for quantification of mosaic c.1872dupAGCTGG can be supplied on request. The assay was first performed using serial dilutions with known proportion of the mutation and the data obtained were plotted to a standard curve. The ratio of normal and mutant DNA being quantified with the GeneMapper version 3.0 program (Applied Biosystems).

3. Results

None of the 10 patients had a family history of neuromuscular disease. Parents of patients 1, 2, 3, 6, 8, 9 and 10 were clinically examined and found not to have muscle weakness, the father of patient 6 also underwent electromyography and it was found to be normal. Parents of patients 4, 5 and 7 could not be examined but they are reported to be healthy.

3.1. Clinical features

Details of the clinical findings are reported in Tables 1 and 2. Age at diagnosis ranged from 5 to 48 years. Age at last examination varied from 11 to 53 years. The onset occurred in the neonatal period or in childhood in 8 patients, while two patients showed signs of muscle weakness after the age of 26 and 35 years respectively. Four patients (#1, 2, 3, 8) had hypotonia and respiratory distress at birth and all but one (#3) required respiratory support for a period varying from few days (#2, 8) to 10 weeks (#1). None of the patients, including those with neonatal onset, had delayed motor milestones, although
walking difficulties, foot drop and difficulty in running and jumping were observed in 8 since early childhood. Seven patients had facial weakness, and all but one had bilateral ptosis manifesting in early infancy (#2, 6) or childhood (#1, 3, 8, 10). Five patients had strabismus, associated with ophthalmoparesis in 4 of the 5. Patients 1 and 8 showed a severe reduction of eye movements since early infancy, patient 8 had mild extraocular muscle involvement manifesting in childhood, whereas the 2 other patients (#4, 5) developed ophthalmoparesis later in adulthood. A high arched palate was observed in seven patients. Three patients (#1, 2, 5) developed mild dysphagia. Muscle wasting involved distal muscles in 5 patients (#4, 5, 6, 7, 10). Generalized muscle atrophy with predominant distal involvement was observed in 3 patients (#1, 2, 3). Muscle weakness mainly involved lower limbs muscles and required ankle-foot orthoses in 3 patients (#2, 3, 7). Neck flexor muscles were severely affected in 5 patients (#1, 2, 3, 8, 10). Two patients (#1, 5) with severe pelvic muscle weakness and marked waddling gait became wheelchair bound in the teenage (#1) and adulthood (#5). Achille’s tendon contractures

Table 1
Clinical and molecular features. Abbreviations used: y, years; m, months; LL, lower limbs; UL, upper limbs; AFOs, ankle-foot orthoses; CNS, central nervous system; NA, not available.

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were observed in 8 patients and 3 of them (#2, 8, 10) required bilateral tenotomy during childhood. In 3 patients (#1, 2, 3) contractures were severe and diffuse involving ankle, knee, hip, elbow and fingers flexors. Jaw muscles contracture with restricted mouth opening was observed in 5 patients (#1, 2, 3, 7, 10), resulting in severe difficulty in food intake in two of them (#1, 2) and inability to intubation in patient 1 with tracheostomy and mechanical ventilation during an episode of acute respiratory failure. Rigid spine and scoliosis were observed in 3 patients (#1, 2, 4). All patients had reduced to absent deep tendon reflexes. Eight patients had pes cavus (#1, 2, 3, 4, 5, 6, 9, 10). Nerve conduction velocity was performed in all patients revealing axonal motor and sensory neuropathy in 2 (#5, 7). EMG showed myopathic changes in 9 patients whereas neurogenic features were observed in patient 10. Pseudomyotonic discharges and fibrillations potentials were found in 3 patients (#2, 3, 7).

None of the patients showed obvious clinical signs of cognitive impairment. Two patients had epilepsy: patient 2 showed generalized seizures since she was 4 years old and she was treated with valproic acid until age of 7 years; patient 10 has partial seizures and she is currently on treatment with carbamazepine. A restrictive respiratory impairment varying from mild to severe degree was observed in all examined patients. Four patients had predicted forced vital capacity (FVC) ranging from 42% to 60% and one of 13%. Two patients were on nocturnal non-invasive ventilation (NNIV). Patient 1, who required the emergency tracheostomy, was gradually weaned to NNIV by the age of 23 years, patient 2 required NNIV since the age of 16 years. Cardiac involvement characterized by tachycardia was observed only in patient 1 starting from the age of 14 years and treated with beta-blockers. Serum CK levels were normal in all patients.

### 3.2. Muscle MRI features

Muscle MRI findings were available in 7 patients (4 children and 3 adults) with different disease severity (Fig. 1). Age at muscle MRI ranged from 8 to 55 years. In the lower legs there was prominent affection of soleus and tibialis anterior muscles followed by peroneal and gastrocnemius muscles. Tibialis posterior muscle was relatively spared except in the most severely affected patients. At thigh level the posterior compartment was more commonly involved, with prominent affection of biceps femoris, semitendinosus and semimembranosus muscles followed by adductor magnus (#6, 3, 5). In patient 2 and 7 there was a more severe involvement of the anterior compartment of the thigh with extensive fatty infiltration of vastus lateralis, vastus intermedius and vastus medialis muscles. Rectus femoris, sartorius and gracilis were relatively spared even in the most severely affected patients. At pelvic level the posterior compartment was more commonly involved, with prominent affection of biceps femoris, semitendinosus and semimembranosus muscles followed by adductor magnus (#6, 3, 5). In patient 2 and 7 there was a more severe involvement of the anterior compartment of the thigh with extensive fatty infiltration of vastus lateralis, vastus intermedius and vastus medialis muscles. Rectus femoris, sartorius and gracilis were relatively spared even in the more severely affected patients (#1, 2, 3, 7, 10). At pelvic level, gluteus maximus was more commonly involved (#2, 3) followed by gluteus medius and minimus. A complete fatty infiltration of gluteal and paraspinal muscles was found in the two more severely affected patients (#1, 5) and
who had lost the ability to walk. Fig. 2 shows a schematic diagram of the selective muscle involvement.

### 3.3. Morphological features

Muscle biopsy was performed in all patients. Ages at biopsy and morphological details are reported in Table 2. All patients showed increased number of centralized nuclei with a percentage of centralization varying from 15% to 90% and predominance and hypotrophy of type 1 fibers. Radial sarcoplasmic strands (RSS) on nicotinamide adenosine dinucleotide–tetrazolium reductase (NADH-TR) staining were detected in all but one patient (#10). Mild endomysial fibrosis was frequently observed even in young patients. In patient 9 (Fig. 3) who had low percentage of centralized nuclei, NADH-TR staining revealed, along with RSS, fibers

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**Fig. 1.** Muscle MRI, T1-weighted images in 7 patients ranging from mild to severe muscular involvement. Age at MRI is reported for each patient. Earliest changes were observed in distal leg muscles with prominent involvement of soleus (#2, 6) and tibialis anterior muscles (#3, 6) followed by peroneal and gastrocnemius muscles (#2, 3). Tibialis posterior muscle was relatively spared except in more severely affected patients (#1, 7). At the level of the thigh posterior compartment was first involved: earliest changes were observed in biceps femoris, semitendinosus and semimembranosus muscles followed by adductor magnus (#6, 3, 5). Patients 2 and 7 showed a more severe involvement of the anterior compartment of the thigh with extensive fatty substitution of vastus lateralis, vastus intermedius and vastus medialis muscles. Rectus femoris, sartorius and gracilis were relatively preserved even in more severely affected patient (#1, 2, 5, 7). At the pelvic level, gluteus maximus was first involved followed by gluteus medius and minimus (#2, 3). A complete fatty infiltration of gluteal and paraspinal muscles was found in more severely affected patients and who had lost the ability to walk (#5, 1).

**Fig. 2.** Schematic diagram of the typical pattern of muscle involvement in DNM2-related CNM. In blue are represented the most affected muscles, in red the relatively spared muscles. A: In the leg there is a prominent involvement of soleus (So) and tibialis anterior (TA). B: In the thigh, biceps femoris (BF), semitendinosus (St) and adductor magnus (AM) are early affected, whereas gracilis (Gr), sartorius (S) and rectus femoris (RF) are relatively spared. C: In the pelvis gluteus maximum (GM) is more affected.
displaying peripheral accentuation of oxidative enzyme activity in an atypical ‘necklace’ appearance. These subsarcolemmal rims were also evident with SDH and COX staining, most of them were slightly basophilic on hematoxylin and eosin (H&E) but were not apparent either on Gomori trichrome (GT) and periodic acid-Schiff (PAS) reagent.

3.4. Genetic features

We identified 6 different mutations, three of which (two missense mutations and one small duplication) have never been reported before (Table 1).

Four patients (#1, 2, 3, 8) showed the same heterozygous mutation in exon 8, c.1102G>A, causing the substitution of glutamate at position 368 to lysin in the middle domain of the protein. In the same domain we identified, in patient 10, the novel c.1124T>G heterozygous missense mutation. The mutation changes a valine at position 375 into glycine and it was excluded in 100 unrelated healthy control subjects. In patients 4 and 7 we identified the heterozygous c.1393C>T mutation in exon 11 in the middle domain. This mutation resulted in a arginine to triptophane substitution at position 465. In patient 9 we found the heterozygous mutation c.1880C>G (p.P627R) in the linker domain between the PH and the GED domains of the protein and resulting in a proline to arginine substitution. In patient 5 we identified a new heterozygous missense mutation in exon 15. The mutation c.1618G>A, changing glutamate 540 to
lysin (p.E540K). The mutation is predicted to be pathogenic by Polyphen-2 and SIFT softwares and it was excluded in 200 control chromosomes. The pathogenetic role is also suggested by the fact that replaces a highly conserved and negatively charged glutamate residue with a positively charged lysine.

While parental samples were not available for patients 4 and 5, both parents of patients 1, 2, 3, 7, 8, 9 and 10 were screened for carrier status and found to be negative, indicating a sporadic occurrence of the mutations in those patients.

In patient 6 we identified a small heterozygous duplication (c.1872dupAGCTGG) of six base pairs in exon 16 (c.1872dupAGCTGG) which led to insertion of a residue of alanine and a residue of glycine in the PH-GED linker domain of the protein (p.624insA-G). The duplication is probably caused by a rearrangement during replication of DNA due to the repetition of the six base pairs sequence (AGCTGG) along the exon 16 which can mediate mitotic sister chromatids ectopic pairing, followed by non allelic homologous recombination. This mutation, which represents the first tandem duplication found in DNM2-CNMs patients, has been excluded in 100 control subjects. While mother of patient 6 did not have the duplication, electropherogram of the DNM2 gene revealed a relatively low level of the mutation in father suggesting a somatic mosaicism (Fig. 4). SNaPshot analysis confirmed the presence of somatic mosaicism in his asymptomatic father, detecting different percentages of the c.1872dupAGCTGG mutation, respectively in DNA from blood (15%), cells from urinary tract (21%), hair (11%) and saliva (19%), Fig. 5.

4. Discussion

Autosomal dominant CNM associated to mutations in DNM2 was first reported by Bitoun and coauthors in 2005 [5] and, more recently, 19 different mutations have been reported in approximately 100 families [9]. In keeping with previous studies we also found that the spectrum of phenotypes ranged from severe forms with neonatal onset to adult onset forms with milder progression [4,5,8,10,14,15]. At variance with previous studies however [8], we observed that none of the patients, even those with neonatal onset, had delayed motor milestones as independent ambulation was always achieved within 18 months. Facial weakness with ptosis and strabismus were always observed at an early stage of the disease whereas ophthalmoparesis frequently occurred later in time. Distal muscles of lower limbs were the first affected muscles in the early stages of the disease and in the milder patients. Contracture of the jaw muscles represents an important clinical aspect already described in patients with neonatal and childhood forms [8,14,16,17]. This feature was common in our series and particularly pronounced in patients carrying the p.E368K mutation. In these patients the restricted mouth opening caused difficulties in food intake. Moreover jaw contracture has to be carefully considered because it can make intubation a challenge in case of acute respiratory failure. Most of our patients showed pes cavus and reduced to absent deep tendon reflexes, and in two of them an axonal sensory-motor neuropathy was detected further confirming the overlap between myopathic and neuropathic phenotype [9,14,18]. All examined patients showed a restrictive ventilatory impairment varying from

Fig. 5. SNaPshotted obtained from blood of patient 6 and from blood, saliva, hair and urine of his asymptomatic father with evidence of somatic mosaicism. The values in the boxes represent the relative peak areas for the mutant sequence.
mild to severe reduction of FVC and impaired cough with necessity of nocturnal assisted ventilation and/or cough machine. One patient had signs of cardiac involvement, that so far has only been reported in a few cases of DNM2-CNMyopathy as late-onset dilated cardiomyopathy, ventricular septal defect or valvular irregularities and non life-threatening arrhythmias [8,9]. Our results confirm the importance of a careful monitoring of pulmonary and cardiac function in these patients [7,8,19]. None of our patients showed cognitive impairment reported so far only in two families carrying the E368Q and R465W mutations [18,20,14] and in one additional patient also showing the R465W mutation [7]. Epileptic seizures, that we found in two patients, have never been described in DNM2-CNMyopathy. Currently we cannot establish that epilepsy is frequently associated to the DNM2 defect, however the possible association should be carefully considered in larger cohorts.

In DNM2 related myopathy muscle imaging has documented a typical pattern of muscle abnormalities [8,10,14,21] characterized by predominant involvement of distal lower leg muscles with early affection of medial gastrocnemius and soleus. At the thigh, hamstring muscles and adductor longus are early involved and at the pelvis, gluteus minimus is more commonly affected. In the present study we describe muscle MRI findings of 7 patients with DNM2 related CNMyopathy that confirm and expand previous findings obtained using computer tomography [14]. In our patients there was a predominant involvement of soleus and tibialis anterior muscles that was observed even in the milder cases. At thigh level, hamstring muscles and adductor magnus were more commonly involved whereas adductor longus, previously reported to be prominently affected [10,21], was relatively spared. As reported, also in our series rectus femoris, sartorius and gracilis were relatively preserved [14]. At pelvic level, gluteus maximus was earlier involved, conversely to what previously reported of a prominent involvement of gluteus minimus [10,14,21]. MRI findings can help in the differential diagnosis with the congenital myopathy related to ryanodine-receptor (RYR1), a condition sharing many clinical and morphological features with DNM2-CNMyopathy including neonatal onset, facial weakness, ophthalmoplegia and type 1 fiber predominance and central nuclei at muscle biopsy [22,23].

Patients with RYR1 mutations have a distinct pattern with a predominant involvement of the anterior thigh compartment, sparing of rectus, adductor longus and gracilis and selective changes in the soleus and lateral gastrocnemius with relative sparing of tibialis anterior within the leg [24]. The pattern observed in our patients was also different from the MRI findings reported in patients with MTM1 mutations, showing a predominant involvement of the posterior thigh compartment, and of the anterior leg compartment and soleus [21,25] and in the rare patients with BIN1 defect [21,26,27], showing a diffuse involvement of all the thigh muscles.

From a genetic point of view all our patients were sporadic cases and de novo appearance of the mutation was verified by sequencing DNA samples of both parents in most of them. This confirms what previously reported in the literature that the majority of patients with DNM2 mutations described so far are sporadic cases with de novo dominant heterozygous mutations [9]. In our series DNA samples of parents were not available in two cases, whereas in patient 6 a somatic mosaicism was detected in the father. In our series the majority of patients had mutations in the middle domain of the protein. The middle domain (MD) is essential for the centrosomal localization [28] and is involved in self-assembly of the molecule [29] and in GTP hydrolysis-induced conformational change of the protein [30]. All the MD mutations identified so far are limited to two adjacent amino acids, Glu368 and Arg369, encoded on exon 8, and a single amino acid Arg465, encoded on exon 11, which constitute the major hot spots for DNM2 mutations [9]. We identified the novel de novo p.V375G mutation in one patient with neonatal onset and moderately severe phenotype further expanding the MD mutational spectrum. In 6 of our patients we found the two known E368K and R465W mutations. The p.E368K change was the most common mutation in our series, having been identified in 4 of 9 patients. An intermediate CNM form has been reported in with early onset and more rapidly progressive course [5,17,8], but severe cases with neonatal onset and delayed motor milestones have also been described [8]. In our series, the p.E368K mutation was associated with presentation at birth, severe progression with loss of independent ambulation from teenage, early involvement of axial muscles and spinal abnormalities, marked contractures and early restrictive respiratory impairment. The p.R465W is the most common DNM2 mutation [9] and reported to be associated with a mild phenotype [5,7,8,17] as confirmed by the 2 patients in our cohort who also had a mild phenotype. Mutations in the pleckstrin homology (PH) and in the PH-GED (GTPase effector) linker domains are frequently associated with severe CMM phenotypes [4,9] and they are also linked to DNM2-related CMT [11,12,31]. In our cohort a novel p.E540K in the PH domain was found in a patient in whom despite the adulthood-onset form with mild myopathy and axonal motor and sensory neuropathy, there was a severe progression with loss of independent ambulation and restrictive respiratory impairment. The two remaining patients have mutations in the linker domain between PH and GED domains. Mutation in the PH-GED linker are rare in DNM2 related CNM as only 6 to 109 CNM-DNM2 families have been reported with mutations in this domain [9].

In patient 6 we identified a small duplication in exon 16 that, to the best of our knowledge, is the first tandem duplication found in DNM2-CNMyopathy. This duplication was also found in his asymptomatic father as somatic
mosaicism. The mutation was detected in blood, urinary tract epithelial cells, hair and saliva with low level ranging from 11% to 21%. Mutation level in muscle could not be assessed, but it should be higher than in the other examined tissues being muscle a more stable tissue. However, as clinical examination and EMG were normal, we can hypothesize that the percentage of the p.624insA-G mutation in the muscle of the father did not reach the threshold level of phenotypic expression. Somatic mosaicism has never been described in CNM patients and only few cases of germinal mosaicism have been reported in X-linked CNM due to mutations in \textit{MTM1} gene [32,33].

The remaining patient carries the p.P627R mutation. This mutation, changing a Proline to Arginine, has recently been described by Böhm et al. [9] in a family with 2 affected members both presenting a childhood onset of symptoms, as observed in our patient, and a relatively mild progression of disease characterized by walking difficulties, reduced vital capacity and diffuse muscle weakness. Although histological and MRI data for these patients are not reported, at the same position the aminocaid change of proline to histidine has also been described by Susman et al. [8] in a boy with a severe neonatal onset congenital myopathy. In our patient muscle biopsy showed atypical “necklace” fibers. Necklace fibers are strongly reactive with oxidative techniques, indicating the presence of mitochondria, but were not evident on GT and PAS, and myonuclei are aligned with the necklace [25]. In the previously reported \textit{DNM2}-CNM patient, muscle fibers showed a necklace-like accentuation on oxidative techniques and PAS but myonuclei were centrally located [35].

In conclusion the findings in our cohort expand the array of genetic mutations adding two novel missense mutations together with the first tandem duplication and reporting the first observation of a somatic mosaicism. Our findings confirm the clinical heterogeneity of CNM associated with \textit{DNM2} mutations and suggest that a progressive course can be observed even in the cases with childhood onset who may have an initial clinical improvement. Our findings also highlight the diagnostic value of muscle MRI in distinguishing \textit{DNM2-CNMs} from other forms of congenital myopathies with similar clinical and morphological features, as \textit{RYR1} and \textit{BIN1} related CNM.

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