Dynamin 2 Mutations Cause Sporadic Centronuclear Myopathy with Neonatal Onset

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We report four heterozygous dynamin 2 (DNM2) mutations in five centronuclear myopathy patients aged 1 to 15 years. They all presented with neonatal hypotonia with weak suckling. Thereafter, their phenotype progressively improved. All patients demonstrated muscle weakness prominent in the lower limbs, and most of them also presented with facial weakness, open mouth, arched palate, ptosis, and ophthalmoparesis. Electrophysiology showed only myopathic changes, and muscle biopsies showed central nuclei and type 1 fiber hypotrophy and predominance. Our results expand the phenotypic spectrum of dynamin 2–related centronuclear myopathy from the classic mild form to the more severe neonatal phenotype.

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The centronuclear myopathies (CNMs)[1] are a heterogeneous group of rare neuromuscular disorders including "myotubular myopathy" for the severe X-linked recessive form (XLMTM) caused by myotubularin mutations[2] and "CNM" for the autosomal forms. Autosomal CNMs comprise a wide spectrum of phenotypes, ranging from severe neonatal to mild late-onset familial forms. The most frequent clinical features are delayed motor milestones, facial and generalized muscle weakness, ptosis, and ophthalmoparesis or ophthalmoplegia.[3,4] The most prominent histopathological features consist of a high frequency of centrally located nuclei in the muscle fibers and predominance and hypotrophy of type 1 fibers.[3–5]

We identified the first mutations responsible for autosomal dominant CNM in the *DNM2* gene encoding dynamin 2 (DNM2),[6] a large GTPase that was mainly implicated in endocytosis and membrane trafficking.[7,8] These first four heterozygous mutations were all restricted to the middle domain of the protein and were identified in families or sporadic cases mostly showing mild, late-onset CNM.[6] Independently, three heterozygous mutations in the pleckstrin homology (PH) domain of DNM2 have also been reported in dominant intermediate Charcot–Marie–Tooth disease type B.[9]

Here, we report five patients with four novel heterozygous mutations in the PH domain of the DNM2 leading to sporadic CNM. In contrast with the previously reported DNM2 mutations, these four mutations are associated with a neonatal onset and a more severe phenotype.

Subjects and Methods

DNM2 Sequencing

The genomic sequence of DNM2 (NT_011295) was used to design intronic primers to amplify the 22 *DNM2* exons, including exons 10bis and 13bis. Polymerase chain reactions and sequencing were done as described previously.[6]

Clinical and Morphological Studies

The clinical data of the five patients were retrospectively reviewed and are summarized in the Table. Motor and sensory nerve conduction studies were performed using standard procedure. Standard histochemical techniques were performed on deltoid or quadriceps muscle biopsies from the five patients (hematein and eosin, myosin adenosine triphosphatase preincubated at pH 9.4 or 4.2, and nicotinamide adenine dinucleotide-tetrazolium reductase staining). Digital photographs were obtained with a Zeiss AxioCam HRc attached to a Zeiss Axioplan Bright Field Microscope (Zeiss, Thornwood, NY). For Patients 1, 2, and 3, muscle samples were also fixed in 2.5% glutaraldehyde and embedded in Polybed 812. Ultrathin sections were stained with uranyl acetate and lead citrate (Reynolds') and examined under a Zeiss 109 electron microscope (Zeiss).

Results

DNM2 Screening

We identified four heterozygous mutations in five CNM patients by sequencing of the coding sequence of *DNM2* gene. The mutations were all located in exon 16: a missense mutation (c.1852G>A) changing alanine 618 to threonine (p.A618T) in Patient 1, a missense mutation

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Table. **Summary of Clinical Data**

Characteristics	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Sex/origin	F/Argentina	F/Argentina	M/Argentina	F/France	F/France
Mutation	p.A618T	p.S619L	p.S619L	p.S619W	p.V625del
Age at last examination, yr	6.5	3.5	1	15	7
At birth					
O_2 therapy/days	Yes/14 days	Yes/4 days	Yes/4 days	No	No
Feeding/nasogastric tube	Yes/few days	Yes/15 days	Yes/up to 1 year	No	No
Motor development					
Head control	12 mo	6 mo	Not acquired at 1 year	2 mo	4 mo
Age sitting	24 mo	8 mo	Not acquired at 1 year	7 mo	9 mo
Age ambulation	30 mo	28 mo	Not acquired at 1 year	14 mo	13 mo
With/without aid	Without aid but orthoses	With aid only (ongoing)		Without aid	Without aid
Other parameters					
Open mouth/arched palate	Yes/Yes	Yes/Yes	Yes/Yes	No/No	—/—
Ptosis	Yes	Yes	Yes	No	Yes
Ophthalmoparesis	Yes, slight	Yes	Yes	No	Yes
Pes cavus	Yes	No	No	Yes	No
Scoliosis	Yes	Yes at 2 yr and 8 mo	No	No	No
Contractures	Yes, Achilles tendon	Yes, Achilles tendon	Yes, Achilles tendon	Yes, Achilles tendon	No
Hyperlaxity		Yes	Yes	No	Yes
Creatine kinase level (UI/L)	92	80	476		37
White blood cells	6,700/mm^3	7,700/mm^3	13,300/mm^3		
Neutrophil counts	54% at 5 yr and 9 mo	45% at 11 mo	30% at 5 mo		
Cardiac function	Mild apex dilatation ventricular function normal	—	Normal	Normal	Normal
NCV, m/sec	—			—	
Left peroneal (age)		48.7 (4 mo) and 60 (2 yr)			64.5 (3 yr)
Left median motor (age)		47.7 (4 mo) and 51.9 (2 yr)			72.1 (3 yr)
Left tibial (age)			22.4 (11 days)		
Left ulnar motor (age)			32.6 (11 days)		
Left median antisensory			32.1		
CMAP, mV	—		—	—	
Peroneal (age)		9.9 (4 mo) and 6.8 (2 yr)			
Median (age)		7.8 (4 mo) and 4.8 (2 yr)			
SNAP, μV			3.3		
Deep tendon reflexes	Present	Present	Weak at 6 mo	Present (2 yr) Absent (8 yr)	Present (3 yr) Absent (7 yr)

Dash denotes no information. NVC = nerve conduction velocities; CMAP = compound muscle action potential; SNAP = sensory nerve action potential.

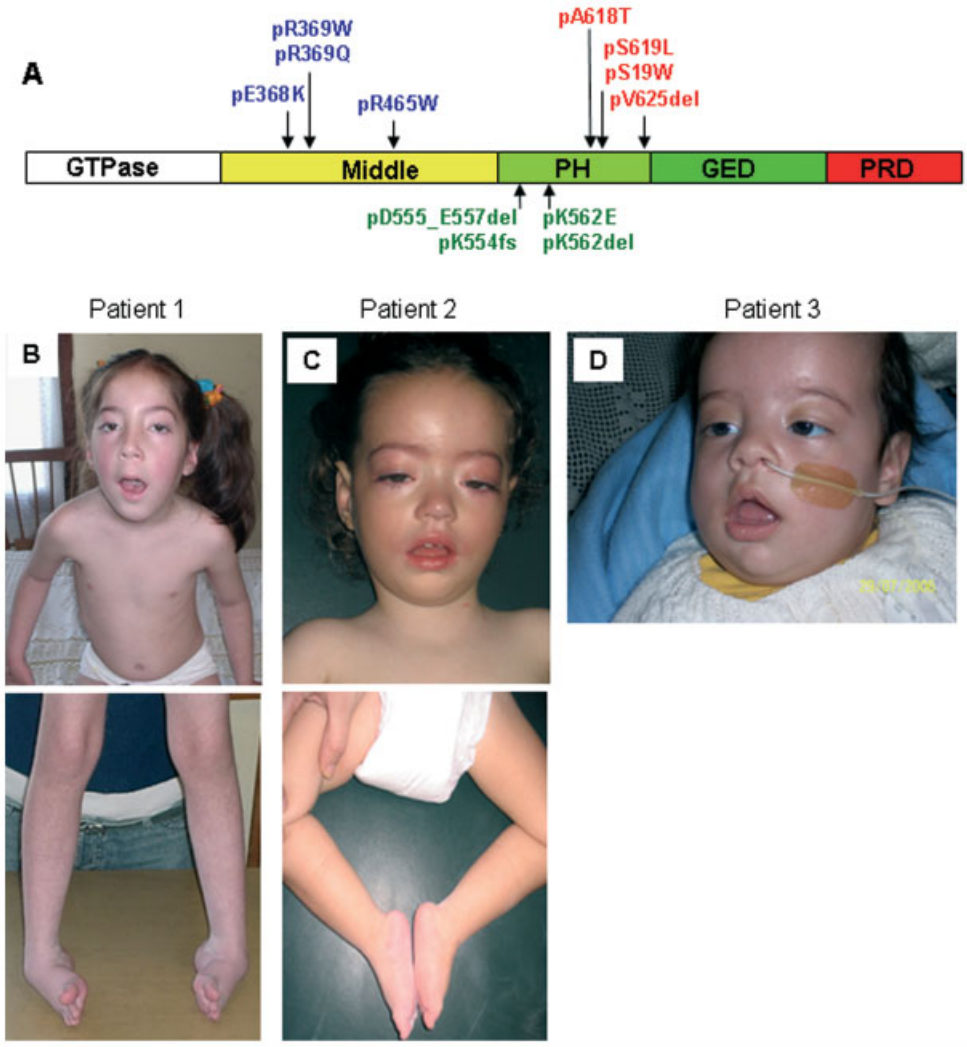

Fig 1. Clinical features of Patients 1, 2, and 3 with early-onset centronuclear myopathy (CNM) with dynamin 2 (DNM2) mutations in the pleckstrin homology (PH) domain. (A) Position of the DNM2 mutations identified in CNM and CMT patients. Mutations are specified on the predicted protein structure including a tripartite GTPase domain (GTPase), a middle domain (Middle), a PH domain, a GTPase effector domain (GED), and a proline-rich domain (PRD). All the variants were numbered according to the same isoform, isoform 1 (GenBank accession number: NP_001005360), because this isoform, which includes the four amino acids GEIL at positions 516 to 519 encoded by exon 13bis, is the major one in human brain, muscle, and primary cultured myoblasts. (B) Patient 1 (mutation p.A618T) at the age of 5 years. (C) Patient 2 (mutation p.S619L) at the age of 2 years. (D) Patient 3 (mutation p.S619L) at the age of 6 months. All patients had generalized hypotonia, bilateral facial weakness, ptosis, and open mouth. A severe ophthalmoparesis was present in Patient 2 (note position of left eye with an internal deviation on top panel). Patient 1 had equinovarus and pes cavus. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

(c.1856C>T) changing serine 619 to leucine (p.S619L) in Patients 2 and 3, a missense mutation (c.1856C>G) changing serine 619 to tryptophan (p.S619W) in Patient 4, and a three-base-pair deletion (c.1873_1875-delGTC) inducing deletion of the valine 625 (p.V625del) in Patient 5. Sequences of DNA samples from parents, available for Patients 1, 2, 3, and 5, were normal, and the paternity was confirmed, indicating de novo mutations. The four mutations were not found in 100 unrelated healthy control subjects. All mutations were detected in three conserved amino acids located in the PH domain of DNM2 (Fig 1A).

Clinical Data

For the five patients, there was no family history of neuromuscular disorder. The clinical features are summarized in the Table. The pregnancies were normal except for the mother of Patient 5, who was affected by pregnancy-induced hypertension. The five patients presented with neonatal hypotonia. None needed respiratory support, although three transiently received oxygen in neonatal period. They all had weak suckling, and three required nasogastric tube for feeding. Three patients presented with marked facial weakness, open mouth, and arched palate in early childhood, and four

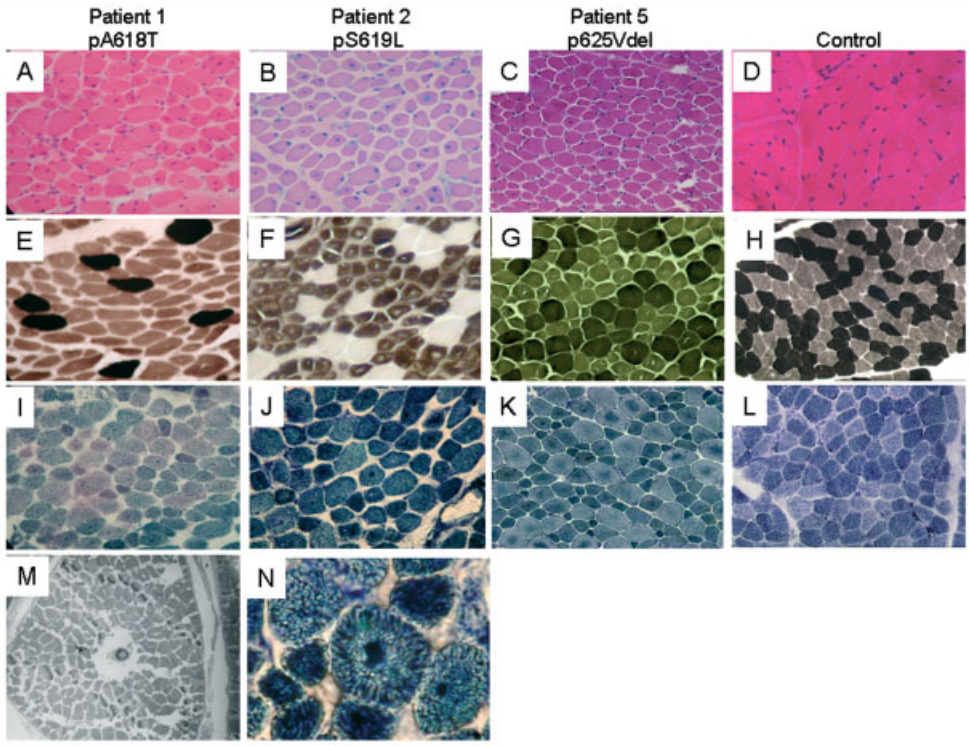

Fig 2. Histological features in dynamin 2 (DNM2)–related centronuclear myopathy (CNM) patient. Hematein and eosin staining (A–D), ATPase preincubated at pH 9.4 (E, G, H) or pH 4.2 (F) staining, and nicotinamide adenine dinucleotide-tetrazolium reductase (NADH-TR) (I–L, N) staining on muscle biopsies from Patients 1 (deltoid biopsy at the age of 5 years), 2 (quadriceps biopsy at the age of 1 year), and 5 (quadriceps biopsy at the age of 2 years), and in a deltoid muscle biopsy performed at the age of 2 years in one healthy patient (control). Representative fields of DNM2-related CNM patients showing internal nuclei (A–C), type 1 fiber predominance and hypotrophy (E–G), and distorted myofibrillary structure (I–K) in comparison with control (D, H, L). (M) Electron microscopy for Patient 1 showed a halo devoid of organelles around central nucleus and an appearance of radiating sarcoplasmic strands. (N) Enlargement of NADH-TR staining for Patient 2 focused on one fiber with radiating sarcoplasmic strands. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

have ptosis and ophthalmoparesis (see Fig 1). Motor milestones were delayed in three patients. Patient 3 was not able to sit or control his head or lift his legs at the age of 1 year, and Patient 2 walked at the age of 28 months but only with aid. Only Patients 4 and 5 reached independent ambulation at a normal age. All demonstrated generalized muscle weakness prominent in lower limbs, and distal limb muscles were more severely affected than proximal muscles. Serum creatine kinase level was slightly increased only in Patient 3. Neutrophil counts available for Patients 1, 2, and 3 were within the reference range for age. There were no cardiac abnormalities on electrocardiogram and echocardiography in Patients 2 to 5, whereas mild apex dilatation was reported in Patient 1. Patients 1, 2, and 3 have no respiratory involvement, but Patients 4 and 5 developed a restrictive respiratory syndrome at the age of 10 and 7 years, respectively.

Patient 2 has been studied twice by electromyography and nerve conduction velocities at the ages of 4 months and 2 years. These showed myopathic changes, normal peroneal and median nerve conduction velocities, and normal compound muscle action potential amplitudes. Patient 3 was similarly evaluated at 11 days after birth. Electromyography showed a myopathic pattern, and motor and sensory nerve conduction studies were normal (see the Table). Patient 5 also showed a myopathic pattern on electromyography at the age of 5 years and had normal motor nerve conduction velocity.

Morphological Data

Quadriceps or deltoid muscle biopsies performed from ages 5 months to 8 years were available for the five patients. All biopsy results showed predominance and hypotrophy of type 1 fibers with central nuclei (Fig 2). Percentage of fibers with central nuclei was 50, 41, 44, and 3%, respectively, for Patients 1, 2, 3, and 5. Nicotinamide adenine dinucleotide-tetrazolium reductase reaction occasionally showed increased oxidative activity around central nuclei. A radial arrangement of sarcoplasmic strands around the central nuclei was observed in only a few fibers, mainly in Patient 2. In the quadriceps biopsy from Patient 3, performed at the age of 5 months, no radial arrangement of sarcoplasmic strands

was observed, even by electron microscopy. Endomysial fibrosis was observed in the biopsies from Patients 1 and 2 (see Fig 2), but there was no evidence of necrosis or regeneration in any of the biopsies.

Discussion

In this study, we identified four novel heterozygous mutations in the PH domain of the DMN2 in young CNM patients who presented with a more severe phenotype than is typical in autosomal dominant CNM.

The four girls and the boy described shared XLMTM features[10,11] such as severe generalized hypotonia and muscle weakness at birth, ophthalmoparesis and facial weakness, and normal serum creatine kinase levels, nerve conduction velocities, and cardiac function. However, in these DNM2-related CNM patients, there were no decreased fetal movements, premature births, or long periods of respiratory insufficiency after birth, although these are often associated with XLMTM. Finally, DNM2-related neonatal CNM is associated with a relatively good prognosis in comparison with XLMTM in which most of patients die during the first year of life from respiratory failure. In our series, only the boy was still very weak at the age of 1 year, whereas the girls progressively became stronger. However, the two oldest patients developed a restrictive respiratory syndrome at the ages of 7 and 10 years and lost deep tendon reflexes.

In these patients, the classic morphological abnormalities described in late-onset CNM patients, especially concerning the radial arrangement of sarcoplasmic strands, were less pronounced. One can hypothesize that their scarcity is probably linked to the young age of the patients. However, despite the diagnosis difficulties, association of central nuclei and type 1 fiber predominance and hypotrophy with the described clinical findings allowed us to identify a group of seven CNM patients, and we found DNM2 mutations in five of them. These results show a genetic heterogeneity in the autosomal neonatal CNM with a high frequency of mutation of the *DNM2* gene and may enable a better selection for direct genetic testing.

Interestingly, three other DNM2 mutations in the PH domain have been reported in dominant intermediate Charcot–Marie–Tooth disease type B,[9] which raises the possibility that these phenotypes may overlap. Previously, we found mild signs of axonal sensorimotor neuropathy in addition to predominant myopathic changes in 4 of 10 patients affected by mild, late-onset CNM harboring mutations in the middle domain of DNM2.[5] In the young patients reported here, the available electrophysiological data only show myopathic changes. It would certainly be of interest to follow the evolution of neurophysiological parameters in these patients to determine a possible progressive peripheral nerve involvement.

In conclusion, we report the first mutations in the PH domain of DNM2 in five patients with a neonatal onset and relatively severe CNM, with the development of a restrictive respiratory syndrome at the end of the first decade of life. These heterozygous de novo mutations enlarge the spectrum of DNM2-related CNM from the classic mild form to include the neonatal phenotype, overlapping with the mild end of the spectrum of XLMTM but with a better prognosis. Our findings show that the *DNM2* gene should be considered in severe sporadic neonatal CNM without MTM1 mutations for which inheritance is impossible to predict.

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