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EXPANDING THE SPECTRUM OF CONGENITAL MYOPATHY LINKED TO RECESSIVE MUTATIONS IN *SCN4A*

Congenital myopathies are phenotypically and genetically heterogeneous.¹ While *SCN4A* mutations were previously described in hypokalemic or hyperkalemic periodic paralysis, paramyotonia, myotonia congenita, or myasthenic syndrome,^{2–6} loss-of-function recessive mutations in *SCN4A* were recently identified in 11 individuals with severe fetal hypokinesia or congenital myopathies.⁷ Among them, 7 died in the perinatal period and 4 had congenital hypotonia, significant respiratory and swallowing difficulties, and spinal deformities.

We report on 3 brothers presenting a peculiar clinical and histopathologic phenotype characterized by facial weakness with ptosis and a mild dystrophic pattern associated with recessive *SCN4A* mutations.

Cases. The 3 probands, including monozygous twins, were born to nonconsanguineous parents (figure, A). Detailed clinical features are presented in table e-1 at Neurology.org. Family history was unremarkable. Both pregnancies were complicated by polyhydramnios and premature birth. All patients had severe neonatal hypotonia and cryptorchidism. The twins showed severe swallowing difficulties, necessitating Nissen fundoplication surgery. Parenteral gastrostomy feeding was set up from the age of 6 and 14 months until 4.5 years of age, respectively. The firstborn developed severe scoliosis. Motor skills were delayed. The patients acquired independent ambulation at 2 years of age. Cognitive development and cerebral MRIs were normal. Clinical examination revealed proximal and axial weakness, elongated face with dolichocephaly, and facial hypotonia with high-arched palate (table e-1). The twins had bilateral nonfluctuant ptosis and global amyotrophy with thin muscular bulk (figure, B–D). There was no myotonia, fluctuating muscle weakness, or fatigability. The 3 brothers experienced a significant clinical improvement over time as they now have normal gait and no problem with jumping or climbing stairs (table e-1). Serum creatine kinase levels were repeatedly normal. The firstborn underwent EMG at age 6 years, revealing a myopathic pattern associated with increased duration of the CMAP and decreased

muscle fiber conduction velocity (rectus femoris muscle [RF]: 1.9 m/s; normal range 2.8–4.1 m/s). Nerve conduction velocities were normal and there was no decrement after repetitive nerve stimulation. EMG, performed at 3 months of age, only showed reduction of muscle fiber conduction velocity (left and right RF: 0.9 m/s and 1.0 m/s, respectively).

Muscle biopsy was performed in the firstborn at age 3 years. Electron microscopy study revealed abnormal subsarcolemmal collection of mitochondria that appeared abnormal in both shape and size (figure, F). Muscle biopsy performed in one of the twins at 5 years of age revealed a mild dystrophic pattern characterized by the presence of scattered necrotic-regenerating fibers presenting immunoreactivity with utrophin, CD68, and MHCd (developmental myosin heavy chain) antibodies (not shown). Mild fiber size diameter variation, increased nuclear internalization, and slight type I fiber predominance were also observed (figure, F).

Exome sequencing was performed for all 6 members of the family. Variants calling and filtering for rare variants compared to ExAC database and an in-house database of 1,550 exomes led to the identification of compound heterozygous mutations in *SCN4A* in the 3 affected members. Neither of those 2 mutations was found in the public variant or the in-house databases. The father carried a missense mutation p.Ser1120Leu (c.3359C > T) affecting a conserved amino acid encoded by exon 18 of *SCN4A* located close to the previously described p.Arg1135Cys found in the adjacent charged transmembrane domain.⁷ The mother carried a single nucleotide exchange in intron 6 (c.1036+1 G > A) in the essential donor splice site. Reverse transcription PCR analysis from patient II-1's muscle showed that this mutation leads to the use of a cryptic donor site in exon 6 and skipping of exon 7, producing a shorter RNA with an in-frame deletion (figure, G).

Discussion. Our study contributes to expanding the phenotypes of congenital myopathies associated with recessive mutations in *SCN4A* by the report of 3 additional cases to the 4 surviving individuals recently described.⁷ The patients were compound heterozygous for 2 novel mutations: an essential splice site variation and a missense variation in *SCN4A*. This led to severe

Supplemental data
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(A) Pedigree of the family with *SCN4A* mutations indicated compared to reference NM_000334. The 3 affected brothers are represented by shaded symbols. Segregation of the mutations was confirmed by Sanger sequencing. Clinical images of patient II-1: (B) bilateral scapular winging, scoliosis, and lumbar hyperlordosis, (C.a) ptosis in patient II-3, (C.b) high-arched palate in patient II-1. (D) Thoracolumbar spine X-ray shows a severe thoracic scoliosis with double curve in patient II-1 (Cobb angles of 59° for the upper curve and 51° for the lower curve). (E) Muscle biopsy analyses (electron microscopy, $\times 10,000$) presence of an abnormal subsarcolemmal collection of mitochondria with irregular shape and size in patient II-1. (F) Light microscopy (hematoxylin & eosin, $\times 20$) shows the presence of scattered basophilic bona fide regenerating fibers in patient II-2 (white arrows). (G) RNA analysis of patient II-1 muscle. (G.a) Agarose gel electrophoresis of reverse transcription PCR amplification product reveals an additional splice product generated by the mutation in intron 6. (G.b) Chromatophorograms show that the impact of the mutation in intron 6 leads to the use of a cryptic donor site in exon 6 and skipping of exon 7, producing a shorter RNA with an in-frame deletion of 183 nt (predicted 61aa deletion in the third extracellular loop of the first transmembrane domain) (left) and the presence of the mutation in exon 18 affecting a conserved amino acid located in the second extracellular loop of the third transmembrane domain (right). (G.c) Scheme of the new splice product created by the mutation in intron 6.

myopathic features at birth with a good prognosis and improvement in the first decade of life. Ptosis was not observed in any member of this first report whereas it was obvious in the twins. It demonstrated that ptosis could be a key feature of this pathology as observed in other congenital myopathies. Moreover, muscle biopsy showed an overlap between myopathic and dystrophic features, broadening the spectrum of histologic traits/specificities associated with mutations in *SCN4A*. This study shows that a multidisciplinary approach including clinical, histologic, electrophysiologic, and genetic description of patients can lead to the identification of the disease-causing mutation and expand the knowledge of myopathy-causing genes such as *SCN4A*.

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