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CORE-ROD MYOPATHY CAUSED BY MUTATIONS IN THE NEBULIN GENE

Nemaline myopathy (NM) and central core disease (CCD), 2 of the most frequent congenital myopathies, are clinically and genetically heterogeneous disorders of skeletal muscle. NM (OMIM 161800) is caused by mutations in at least 6 different genes encoding thin filament proteins of the striated muscle sarcomere: skeletal alpha-actin (*ACTA1*), nebulin (*NEB*), beta-tropomyosin (*TPM2*), slow alpha-tropomyosin (*TPM3*), slow skeletal muscle troponin-T (*TNNT1*), and cofilin-2 (*CFL2*). CCD (OMIM 117000) is mainly caused by mutations in the gene encoding the skeletal muscle ryanodine receptor (*RYR1*), a Ca^{2+} channel involved in excitation-contraction coupling. Congenital myopathies with the combination of distinct “cores” and “rods” in the same muscle fibers have been described in a small number of cases. In 2 families mutations have been identified in *RYR1*^{1,2} and in others, linkage has been found to a locus on chromosome 15.³

Here we report a patient with recessively inherited core-rod myopathy caused by compound heterozygous mutations in *NEB*. This observation broadens the spectrum of genes involved in core-rod myopathies.

Case report. The patient is a 27-year-old white man. He presented at birth with generalized hypotonia and required immediate intubation and resuscitation. Spontaneous movements were very poor and he had seizures from the first week. Restrictive respiratory insufficiency led to continuous mechanical ventilation via tracheotomy at the age of 2 years. The patient did not acquire independent walking but was able to take a few steps with orthosis at the age of 4; diffuse muscle weakness with axial predominance was observed. Electromyography showed myopathic patterns and normal motor and sensory responses. Serum creatine kinase levels were normal. The patient developed multiple joint contractures and spinal fusion was performed at 14 years due to scoliosis. At adult age, he was quadriplegic and had mild facial weakness but no ocular involvement. No cardiac symptoms were observed. Whole-body MRI assessment showed no major abnormalities in CNS, and

diffuse muscle involvement with predominance in axial and proximal muscles. Lateral and medial gastrocnemius, temporal and medial pterygoid muscles were selectively preserved (figure, A, B-a, and B-b). No muscles were selectively preserved at the thigh level.

Deltoid muscle biopsies performed at the ages of 2 and 7 years showed numerous fibers with distinctive clusters of rods and central well-delineated cores (in type 1 fibers), and type 1 fiber predominance. Electron microscopy revealed rods with the characteristic striation and well-defined structured cores, with some focal sarcomeric disorganization, and depletion of mitochondria; cores extend almost along the full length of the fibers (figure, C–F). Molecular genetic analysis using DNA extracted from blood showed no mutations in exons of *ACTA1* and *RYR1* genes. Subsequently, 2 different duplications were identified in *NEB*: g.195187_195188dupAC in exon 140 and g.234878_234881dupTCAA in exon 171. Both are expected to cause truncation of the nebulin protein.

Discussion. We report a patient with congenital myopathy with cores and rods associated with *NEB* mutations. He was severely hypotonic since birth and required continuous mechanical ventilation. Perinatal complications were initially suspected due to the relative sparing of facial muscles and the presence of seizures in the first week, but complementary tests ruled out major medullar or CNS damage. Overall, muscle biopsies showed signs of congenital myopathy with characteristic cores with defined borders and clusters of rods; cores did not appear significantly different from that observed in CCD. No signs suggesting neurogenic damage as the cause of the sarcomeric rearrangement were observed. Muscle MRI findings in the leg were compatible with those observed in *NEB*-related nemaline myopathy.⁴

Molecular analysis demonstrated the presence of compound heterozygous mutations in *NEB*. Six other core-rod myopathy patients with no known genetic cause were analyzed for mutations in the same regions of *NEB*, but no mutations were found. Among the patients with NM caused by mutations in *NEB*, none have the same combination of muta-

Clinical picture and muscle MRI of the patient at 27 years of age: the face is only mildly affected (A); severe generalized weakness and amyotrophy with multiple joint contractures is present (B-a); T1-weighted axial view of the leg shows severe abnormal signal in soleus and anterior and lateral compartments, while gastrocnemius muscles are strikingly spared (So = soleus; Gm = medial gastrocnemius; Gl = lateral gastrocnemius; TA = tibialis anterior) (B-b). Serial transverse muscle sections of muscle biopsy taken at the age of 7 years showed 2 distinctive morphologic abnormalities: with the Gomori trichrome stain, clusters of rods in a large number of fibers (fibers with high magnification in the inset) (B-b, C), and with oxidative reactions (NADH-TR), large well-delineated cores (E). On electron microscopy, in transverse sections the rods showed characteristic striation and had the same electron density as the Z line of adjacent sarcomeres; they were separated from the myofibrils and appeared randomly or in clusters, under the sarcolemma or dispersed in the sarcoplasm (D). In longitudinal sections, the cores had a structured appearance with jagged Z disks and absence of mitochondria. The structured cores spanning almost the entire fiber length showed maintained basic sarcomeric architecture, although some focal loss of cross-striations (foci of Z-line streaming) that extended few sarcomeres coexisted within the cores (F).

tions as the patient described here. One patient with a severe form of NM associated with unusual clinical features is compound heterozygous for the same duplication in exon 171, but the second mutation is a nonsense mutation in exon 119. Frameshift mutations in exon 171 have also been observed in patients with mild and typical NM.⁵ Exon 171 is alternatively spliced and mutations would only affect those nebulin isoforms expressing this exon. Exon 140 is constitutively expressed and the frameshift-causing duplication would result in truncation of all nebulin isoforms. We have not identified any other mutation in this exon. The reason for the formation of cores in addition to rods in this patient's muscle is unknown.

After the descriptions of nemaline myopathy⁶ and distal nebulin myopathy,⁷ core-rod myopathy is the third entity to be associated with mutations in *NEB*.

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1. Scacheri PC, Hoffman EP, Fratkin JD, et al. A novel ryanodine receptor gene mutation causing both cores and rods in congenital myopathy. *Neurology* 2000;55:1689–1696.
2. Monnier N, Romero NB, Lemale J, et al. An autosomal dominant congenital myopathy with cores and rods is associated with a neomutation in the RYR1 gene encoding the skeletal muscle ryanodine receptor. *Hum Mol Genet* 2000;9:2599–2608.
3. Gommans IM, Davis M, Saar K, et al. A locus on chromosome 15q for a dominantly inherited nemaline myopathy

with core-like lesions [erratum in 2003;126:2115]. *Brain* 2003;126:1545–1551.

4. Jungbluth H, Sewry CA, Counsell S, et al. Magnetic resonance imaging of muscle in nemaline myopathy. *Neuromuscul Disord* 2004;14:779–784.
5. Lehtokari VL, Pelin K, Sandbacka M, et al. Identification of 45 novel mutations in the nebulin gene associated with autosomal recessive nemaline myopathy. *Hum Mutat* 2006;27:946–956.
6. Pelin K, Hilpela P, Sewry C, et al. Mutations in the nebulin gene associated with autosomal recessive nemaline myopathy. *Proc Natl Acad Sci USA* 1999;96:2305–2310.
7. Wallgren-Pettersson C, Lehtokari V-L, Kalimo H, et al. Autosomal recessive distal myopathy caused by missense mutations in the nebulin gene. *Brain* 2007;130:1465–1476.

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LINGO1 VARIANT INCREASES RISK OF FAMILIAL ESSENTIAL TREMOR

Essential tremor (ET), a neurologic disorder characterized by postural and action tremor, is one of the most common adult-onset motor disorders.^{1,2} While linkage regions have been identified in Icelandic and North American families, the causative gene remains elusive.^{1–4} Recently, a sequence variant (rs9652490 G allele) of the *LINGO1* gene was found to be associated with ET in the first genome-wide association study in European and American populations.⁵ Here we determined the linkage disequilibrium (LD) of single nucleotide polymorphisms (SNPs) 100 kb up/downstream of the implicated *LINGO1* SNP and analyzed the SNP in a case control study in an Asian population.

We included consecutive patients with ET who presented to a tertiary referral center and examined by movement disorders neurologists following a methodology as previously described.⁶ We followed the diagnostic criteria of classic ET based on the recommendations of the Consensus Statement of the Movement Disorders Society in 1998.⁷ Patients with at least one affected first-degree relative were classified as familial ET. Controls of similar gender, race, and age as ET patients and physically examined by the authors at the Health Screening Unit of the same hospital were included. Informed consent from all the study subjects was taken and the work received approval from the institutional ethics committee.

The *LINGO1* SNP (rs9652490) was screened on MALDI-TOF mass spectrometry using the Sequenom MassARRAY^{TM} system (San Diego, CA) (details described in appendix e-1 on the *Neurology*® Web site at www.neurology.org).

A total of 923 subjects (733 controls and 190 ET) examined by the authors were included in the analysis. The median age of the controls (56.7 years) was 15 and 4 years older than the median age at onset (41.0 years) and age of patients with ET (52.6 years).

A total of 75 (39.5%) patients with ET reported a positive family history in at least 1 first-degree relative. There was a trend toward a higher frequency of the allele G of marker rs9652490 in ET (0.263 vs 0.218, OR = 1.28, $\chi^2 = 3.29$, $p = 0.068$). For the genotypic test, OR_{AG} = 0.84, OR_{GG} = 2.43, $\chi^2 = 13.53$, $p = 0.001$, and for recessive inheritance (allele G – [GG vs AA+AG]), $\chi^2 = 12.72$, $p = 0.00036$, OR_G = 2.58. The population attributable risk of genotype GG = 18.8%. There is no evidence of heterogeneity between the results from our population and genotype data from the American and European populations ($I^2 = 0$, $p = 0.66$). A combined meta-analysis revealed an OR = 1.48, $p = 9.1 \times 10^{-10}$ (table). Interestingly, analysis of only those with familial ET revealed a much more robust association (0.32 vs 0.22, OR = 1.69, $\chi^2 = 7.27$, $p = 0.007$). For the genotypic test, OR_{AG} = 1.23, OR_{GG} = 3.26, $\chi^2 = 10.55$, $p = 0.005$. Analysis of sporadic ET did not reveal any association (0.225 vs 0.218, OR = 1.04, $p = 0.8$). LD variation surrounding this SNP was largely similar between Caucasians and Asians (figure e-1). The maximum F_{ST} (fixation index) value for the SNP is only 0.0002 or 0.2% between Chinese (19.8%) and Germans (23.3%).

We have found negligible LD variation involving multiple SNPs 100 kb up/downstream of the functional *LINGO1* SNP (rs9652490) between Asians and Caucasians. The frequency of the G allele (0.32) in our Asian patients with familial ET and the OR of 1.66 were practically identical to the discovery data set from Iceland, but higher than most of the other follow-up datasets (table). These studies have either analyzed both familial and sporadic ET as a group or did not provide information on the specific group of patients with ET (table e-1). The similarity of our result with the Icelandic discovery dataset could be explained by the fact that the majority (69.4%) of their patients with ET reported a positive family history. It would be interesting to determine how the

Supplemental data at
www.neurology.org

Table	Summary of published literature					
	Controls		Cases		OR (95% CI)	p Value
	No.	Frequency	No.	Frequency		
Discovery (5)						
Iceland	14,378	0.230	452	0.329	1.63 (1.35, 1.97)	3.0×10^{-7}
Follow-up (5)						
Austria	342	0.193	77	0.292	1.73 (1.15, 2.59)	0.0082
Germany	176	0.233	69	0.297	1.39 (0.89, 2.17)	0.15
United States	611	0.222	119	0.273	1.32 (0.92, 1.90)	0.14
Iceland	290	0.224	35	0.271	1.29 (0.71, 2.36)	0.41
Asian data						
Singapore	734	0.219	190	0.263	1.27 (0.98, 1.65)	0.068
All combined	15,797	—	752	—	1.48 (1.30, 1.67)	9.1×10^{-10}

OR = odds ratio; CI = confidence interval.

overall effect size of the association will be affected if only familial cases are examined in Caucasians. In the *LINGO1* discovery study,⁵ the 14,393 Icelandic controls were not screened for ET and in the 2 follow-up studies in Austria and Germany, the median age of controls was about 20–25 years younger than patients with ET. It is unclear how these would have influenced the reported effect size difference.

We have demonstrated negligible LD variation in the vicinity of the *LINGO1* SNP (rs9652490) between Asians and Caucasians. This sequence variant (rs9652490 G allele) is associated with risk of familial ET. An individual with two G alleles has more than 3 times risk of having ET. Replication studies in other populations are warranted.

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1. Deng H, Le W, Jankovic J. Genetics of essential tremor. *Brain* 2007;130:1456–1464.
2. Louis ED. Essential tremor. *Lancet Neurol* 2005;4:100–110.
3. Higgins JJ, Pho LT, Nee LE. A gene (ETM) for essential tremor maps to chromosome 2p22-p25. *Mov Disord* 1997;12:859–864.
4. Tan EK, Schapira AH. Hunting for genes in essential tremor. *Eur J Neurol* 2008;5:889–890.
5. Stefansson H, Steinberg S, Petursson H, et al. Variant in the sequence of the *LINGO1* gene confers risk of essential tremor. *Nat Genet* 2009;41:277–279.
6. Tan EK, Prakash KM, Fook-Chong S, et al. *DRD3* variant and risk of essential tremor. *Neurology* 2007;68:790–791.
7. Deuschl G, Bain P, Brin M. Consensus statement of the Movement Disorder Society on Tremor: Ad Hoc Scientific Committee. *Mov Disord* 1998;13 suppl 3:2–23.