The *de novo* missense mutation N117S in skeletal muscle α-actin 1 causes a mild form of congenital nemaline myopathy

LIU YANG[1], PING YU[2], XIANG CHEN[1] and TAO CAI[2,3]

[1]Physical Medicine and Rehabilitation Center, The Second Affiliated Hospital and Yuying Children's Hospital, Wenzhou Medical University; [2]Institute of Genomic Medicine, Wenzhou Medical University, Wenzhou, Zhejiang 325027, P.R. China; [3]Experimental Medicine Section, National Institute of Dental and Craniofacial Research, National Institutes of Health, Bethesda, MD 20892, USA

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Abstract. Nemaline myopathy (NM) constitutes a spectrum of primary skeletal muscle disorders, the diagnosis of which is based on muscle weakness and the visualization of nemaline bodies in muscle biopsies. Mutations in several NM causal genes have been attributed to the majority of NM cases, particularly mutations in nebulin and skeletal muscle α-actin 1 (*ACTA1*), which are responsible for ~70% of cases; therefore, a genetic diagnostic strategy using targeted gene sequencing may potentially improve the diagnosis of suspected NM. The present study identified a *de novo* mutation in *ACTA1* (c.350A>G; p.Asn117Ser) in a Chinese patient using target-capture sequencing of a panel containing 125 known causal genes for inherited muscle diseases. Clinical analyses revealed that the case described in the present study exhibited a relatively mild phenotype with regards to muscle weakness, as compared with more severe phenotypes reported in several other patients with the same mutation, thus suggesting the existence of genetic modifiers. In conclusion, this approach may be helpful for the identification of clinically undiagnosed patients with highly heterogeneous disorders.

Introduction

Nemaline myopathy (NM) is a genetically and clinically heterogeneous form of myopathy, which has an incidence rate of ~1/50,000 live births worldwide (1). In China, <50 cases, as determined by clinical analyses and pathohistological examinations, have been reported in the literature since the first case in 1990 (2-6). In a previous study, in 4,127 patients with suspected myopathy who received muscle biopsies, the occurrence of NM was only ~0.29% (28 cases) (2), thus suggesting that NM is a rare disorder in Chinese populations. To date, at least eight genes, including nebulin (*NEB*), skeletal muscle α-actin 1 (*ACTA1*), cofilin-2, troponin T1, β-tropomyosin, slow-muscle α-tropomyosin, kelch repeat and BTB domain-containing 13 and kelch-like family member 40, have been reported to cause NM (7). The majority of these genes encode sarcomeric thin filament proteins. Among these causal genes, ~50% of the autosomal recessive mutations associated with NM were attributed to the *NEB* gene, whereas 20% of the associated autosomal dominant or autosomal recessive mutations were attributed to the *ACTA1* gene (7). To gain insight into the pathogenesis of NM, several animal models, including mouse (8) and zebrafish (9), have been established, and various therapeutic approaches are under investigation (10).

In addition to clinical studies regarding NM, mechanistic studies regarding molecular and genetic pathogenesis have been conducted (11). However, only two compound heterozygous variants (c.9052 G>A, c.24579G>A) in the *NEB* gene have been identified to date in two patients with NM in a Chinese family (6). No mutations in the other aforementioned genes, to the best of our knowledge, have been detected in patients with NM in China. Although genetic testing is recommended in patients with congenital myopathy, its clinical application has been limited by the cost and time-consuming nature of conventional sequencing. The present study adapted the targeted exome capture technique for rapid sequencing of potential mutations in a patient with congenital myopathy. The present study identified a *de novo* missense mutation in *ACTA1* in a Chinese Han patient with a milder NM phenotype, and also compared the clinical manifestations in previously reported cases with the same mutation.

Materials and methods

Patient information. The patient was an 11-year-old girl presenting with muscle weakness, she was the second child

Correspondence to: Dr Xiang Chen, Physical Medicine and Rehabilitation Center, The Second Affiliated Hospital and Yuying Children's Hospital, Wenzhou Medical University, 109 Xueyuan Western Road, Wenzhou, Zhejiang 325027, P.R. China
E-mail: chenxiangnj2005@aliyun.com.cn

Dr Tao Cai, Experimental Medicine Section, National Institute of Dental and Craniofacial Research, National Institutes of Health, 30 Convent Drive, Bethesda, MD 20892, USA
E-mail: tcai@mail.nih.gov

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Table I. Phenotypic comparison of patients with the N117S skeletal muscle α-actin 1 mutation.

Author (year)	Patient	Age	Type of mutation	Phenotype	Reference
Nowak et al (1999)	#7 (mother), family 6	33 (deceased)	Autosomal dominant	Typical NM	(12)
	#8 (child), family 6	18 (deceased)	Autosomal dominant	Typical NM	(12)
	#9 (child), family 6	3 (deceased)	Autosomal dominant	Severe NM	(12)
Yang et al (2016)	Proband (sporadic case)	11 (alive)	*De novo* dominant	Mild NM could walk freely, could not jump	Present study

NM, nemaline myopathy.

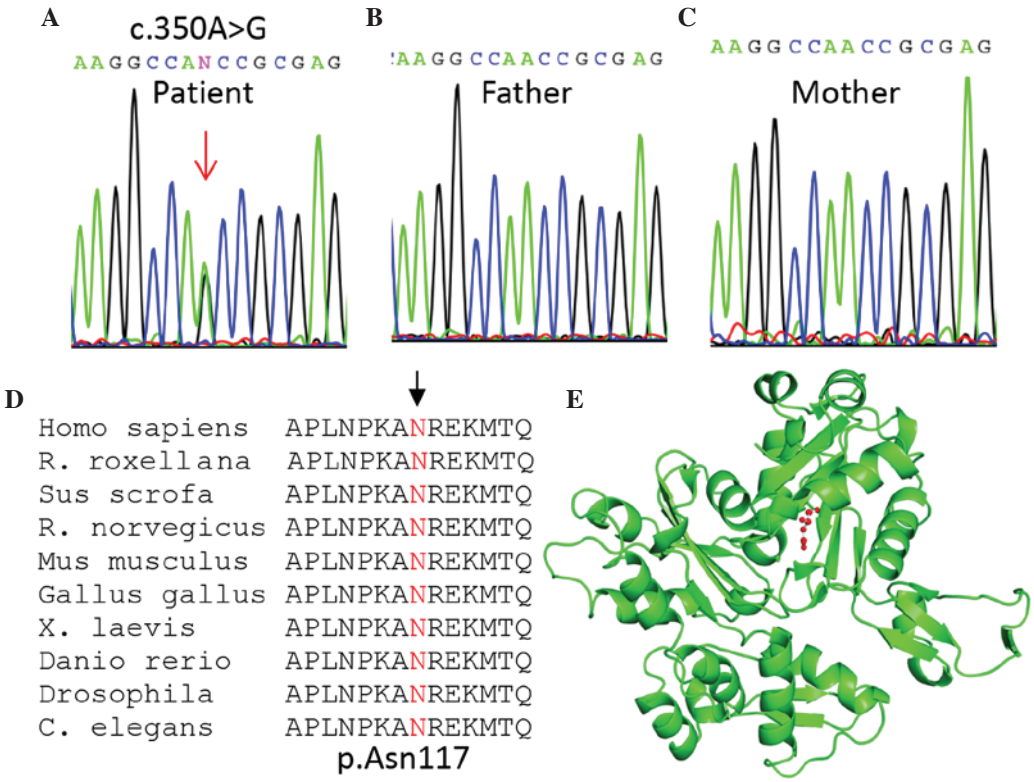

Figure 1. A causal mutation of skeletal muscle α-actin 1 identified in a Chinese patient. (A-C) *De novo* missense mutation was confirmed in the patient by Sanger sequencing. (D) The N117 residue is highly evolutionarily conserved, as shown by multiple sequence alignment. (E) The 3D structural model is established using PyMOL Molecular Graphics System. Position of N117 (in red) in the three dimensional representation of the α-actin monomer (Protein Database Bank code 1ATN). The N117 was designated as N115 in 1ATN since the first two amino acids of the protein were removed in the 3D structural model.

born to a healthy nonconsanguineous 24-year-old mother and 27-year-old father. There was no family history of neuromuscular or other inherited diseases. Creatine kinase measurements and electromyography assessment were performed routinely in the hospital. Written informed consent was obtained from the patient's parents. The present study was approved by the ethics committee of Wenzhou Medical University (Wenzhou, China).

Targeted gene exome sequencing. Genomic DNA was isolated from the peripheral blood leukocytes of the patient and her parents using Wizard® Genomic DNA Purification kit (Promega Corporation, Madison, WI, USA). The BestSeq target-capture sequencing panel, which contains 125 known causal genes for inherited muscle diseases, was used to analyze the DNA of the patient and her parents (conducted by Huakang Gene Institute, Beijing, China). Polymerase chain reaction and Sanger sequencing using primers for the ACAT1 N117S mutation (forward, CGCGTAGCCCTCATAAATGG; reverse, CGACGAGGCTCAGAGCAAGA) were also performed by Huakang Gene Institute to confirm the mutation.

Results

The patient was found to have delayed gross motor skills. She could walk independently at two years old and continued to exhibit progressive improvement in motor development

during childhood. Her fine motor activities were adequate; however, her knee jerk reaction and ankle reflex could not be obtained. In addition, her biceps and triceps reflexes could not be obtained. No positive pyramidal sign was detected. The patient had difficulty walking upstairs, and could not jump. No sensory disturbance was identified. Her intellectual capacity was equivalent to that of an 11-year-old child. Her height and body weight were in the normal range. Furthermore, an electromyogram indicated possible myogenic damage. Her serum creatine kinase level was 25 U/l, which is slightly lower than the normal reference range (26-140 U/l).

The clinical manifestations of this patient suggested an undiagnosed myopathy. To identify potential mutation(s), either a *de novo* mutation or a recessive mutation associated with muscular diseases, BestSeq target-capture sequencing was conducted using DNA samples from the patient and her parents. In the panel of 125 known causal genes for inherited muscle diseases, a missense mutation in exon 3 of the *ACTA1* gene at chromosome 1q42.13 (NM_001100.3) was identified. In addition, the mutation (c.350A>G; p.Asn117Ser) was confirmed to be a *de novo* mutation since it was only detected in the patient (Fig. 1A), and not her parents (Fig. 1B and C).

The identified mutation was predicted to be damaging or disease-causing using three commonly used tools: SIFT (http://sift.jcvi.org/; affecting protein function with a score of 0.00), PolyPhen-2 (http://genetics.bwh.harvard.edu/pph2/; possibly damaging, with a score of 0.921), and MutationTaster (http://www.mutationtaster.org/; disease-causing with a probability score of 0.99999). The N117 residue (as indicated by the arrow; Fig. 1D) is located in the nucleotide-binding domain of the sugar kinase/heat shock protein 70/actin superfamily, which is evolutionarily conserved from human to *Caenorhabditis elegans* as shown by multiple sequence alignment (Fig. 1D). Based upon three-dimensional structural analysis using PyMOL Molecular Graphics system, version 1.3 (www.pymol.org), N117 (designated as N115 since the first two amino acids were removed in the crystal structure) was located near the nucleotide-binding cleft and was thus predicted to affect nucleotide binding (Fig. 1E).

Notably, the same mutation (p.N117S) in the *ACTA1* gene has previously been identified as a pathogenic locus (rs121909520) in Australian patients with an autosomal dominant inheritance pattern of NM (12-14), and was associated with a typical or severe phenotype (Table I). The three affected individuals succumbed at 36, 18, and 3 years of age, respectively. Conversely, the patient presented in the current study exhibited a much milder phenotype (Table I), thus suggesting a genetic heterogeneity or the existence of genetic modifiers.

Discussion

NM (OMIM, #161800) is a novel form of congenital myopathy that was described in 1963 (15), and is characterized by abnormal thread- or rod-like structures in muscle fibers upon histological examination. In the Human Gene Mutation Database professional database (http://www.hgmd.cf.ac.uk/ac/index.php), a total of 196 mutations in the *ACTA1* gene, including 181 missense mutations, 10 indels and five splicing events, have been curated. *ACTA1* mutations (OMIM, *102610) have been reported to induce several subtypes of myopathy, including nemaline myopathy 3 and congenital myopathy with excess of thin myofilaments or cores (OMIM, #161800), and congenital myopathy with fiber-type disproportion 1 (OMIM, #255310).

In general, two clinical groups of NM can be readily distinguished: i) Typical NM is the most common form (46% of all NM cases), presenting as infantile hypotonia and muscle weakness; and ii) severe NM, which is observed in 16% of NM cases, and is characterized by an absence of spontaneous movement or respiration at birth, arthrogryposis, and mortality in the first months of life (16). Mutations in the *ACTA1* gene account for ~20% of typical NM cases and ~50% of severe NM cases (16). In the present study, NM was derived from a *de novo* mutation, and the patient presented with non-progressive muscle weakness. Compared with previously reported patients with the same mutation, the phenotype of the patient described in the present study appears much milder.

The N117S mutation is mostly associated with milder, typical NM since it is located near the nucleotide-binding cleft, and is predicted to affect nucleotide binding, a defect that may result in a decreased polymerization propensity (14). It has also been observed that the N117S mutant was not readily integrated into stress fibers, since the mutant produced a diffuse cytoplasmic staining in the majority of undifferentiated myoblasts (17). In addition, compared with the wild-type gene, the N117S mutant has been reported to have reduced copolymerization capacity with wild-type actin (18).

In conclusion, to the best of our knowledge, the present study is the first to identify a pathogenic mutation in *ACTA1* in a Chinese patient with suspected myopathy. The present study benefited from exome-target capture and next-generation sequencing technology, as recently applied in NM and other inherited diseases (19-22). Further application of this approach will be helpful for the identification of several undiagnosed patients with this type of highly heterogeneous disorder.

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References

1. Wallgren-Pettersson C, Kääriäinen H, Rapola J, Salmi T, Jääskeläinen J and Donner M: Genetics of congenital nemaline myopathy: A study of 10 families. J Med Genet 27: 480-487, 1990.
2. Yin X, Pu CQ, Wang Q, Liu JX and Mao YL: Clinical and pathological features of patients with nemaline myopathy. Mol Med Rep 10: 175-182, 2014.
3. Han Y, Zheng H and Ding S: Delayed onset of nemaline myopathy: A case report. Chin Med J (Engl) 116: 798-800, 2003.
4. Jiang H, Xiao B, Jia DD, Zhang N, Xu XP and Tang BS: Clinical and pathologic analysis of an autosomal recessive kindred with nemaline myopathy. Zhonghua Yi Xue Za Zhi 89: 3316-3319, 2009 (In Chinese).
5. Jiang C, Wang J and Lu H: Clinical and pathological features of childhood-onset nemaline myopathy: A report of four cases. Case Rep Med 2012: 203602, 2012.
6. Chen Z, Wang JL, Tang BS, Sun ZF, Shi YT, Shen L, Lei LF, Wei XM, Xiao JJ, Hu ZM, *et al*: Using next-generation sequencing as a genetic diagnostic tool in rare autosomal recessive neurologic Mendelian disorders. Neurobiol Aging 34: 2442.e11-e17, 2013.
7. Romero NB, Sandaradura SA and Clarke NF: Recent advances in nemaline myopathy. Curr Opin Neurol 26: 519-526, 2013.

8. Garg A, O'Rourke J, Long C, Doering J, Ravenscroft G, Bezprozvannaya S, Nelson BR, Beetz N, Li L, Chen S, *et al*: KLHL40 deficiency destabilizes thin filament proteins and promotes nemaline myopathy. J Clin Invest 124: 3529-3539, 2014.
9. Sztal TE, Zhao M, Williams C, Oorschot V, Parslow AC, Giousoh A, Yuen M, Hall TE, Costin A, Ramm G, *et al*: Zebrafish models for nemaline myopathy reveal a spectrum of nemaline bodies contributing to reduced muscle function. Acta Neuropathol 130: 389-406, 2015.
10. Wallgren-Pettersson C, Sewry CA, Nowak KJ and Laing NG: Nemaline myopathies. Semin Pediatr Neurol 18: 230-238, 2011.
11. Tian L, Ding S, You Y, Li TR, Liu Y, Wu X, Sun L and Xu T: Leiomodin-3-deficient mice display nemaline myopathy with fast-myofiber atrophy. Dis Model Mech 8: 635-641, 2015.
12. Nowak KJ, Wattanasirichaigoon D, Goebel HH, Wilce M, Pelin K, Donner K, Jacob RL, Hübner C, Oexle K, Anderson JR, *et al*: Mutations in the skeletal muscle alpha-actin gene in patients with actin myopathy and nemaline myopathy. Nat Genet 23: 208-212, 1999.
13. Ilkovski B, Cooper ST, Nowak K, Ryan MM, Yang N, Schnell C, Durling HJ, Roddick LG, Wilkinson I, Kornberg AJ, *et al*: Nemaline myopathy caused by mutations in the muscle alpha-skeletal-actin gene. Am J Hum Genet 68: 1333-1343, 2001.
14. Sparrow JC, Nowak KJ, Durling HJ, Beggs AH, Wallgren-Pettersson C, Romero N, Nonaka I and Laing NG: Muscle disease caused by mutations in the skeletal muscle alpha-actin gene (ACTA1). Neuromuscul Disord 13: 519-531, 2003.
15. Shy GM, Engel WK, Somers JE and Wanko T: Nemaline Myopathy. A new congenital myopathy. Brain 86: 793-810, 1963.
16. North KN and Ryan MM: Nemaline myopathy. In: GeneReviews® [Internet]. Pagon RA, Adam MP, Ardinger HH, Wallace SE, Amemiya A, Bean LJH, Bird TD, Fong CT, Mefford HC, Smith RJH and Stephens K (eds.). University of Washington, Seattle, WA, 1993-2016.
17. Bathe FS, Rommelaere H and Machesky LM: Phenotypes of myopathy-related actin mutants in differentiated C2C12 myotubes. BMC Cell Biol 8: 2, 2007.
18. Costa CF, Rommelaere H, Waterschoot D, Sethi KK, Nowak KJ, Laing NG, Ampe C and Machesky LM: Myopathy mutations in alpha-skeletal-muscle actin cause a range of molecular defects. J Cell Sci 117: 3367-3377, 2004.
19. Sambuughin N, Zvaritch E, Kraeva N, Sizova O, Sivak E, Dickson K, Weglinski M, Capacchione J, Muldoon S, Riazi S, *et al*: Exome analysis identifies Brody myopathy in a family diagnosed with malignant hyperthermia susceptibility. Mol Genet Genomic Med 2: 472-483, 2014.
20. Langouet M, Siquier-Pernet K, Sanquer S, Bole-Feysot C, Nitschke P, Boddaert N, Munnich A, Mancini GM, Barouki R, Amiel J and Colleaux L: Contiguous mutation syndrome in the era of high-throughput sequencing. Mol Genet Genomic Med 3: 215-220, 2015.
21. Yu P, Cui Y, Cai W, Wu H, Xiao X, Shao Q, Ma L, Guo S, Wu N, Jin ZB, *et al*: Lysosomal storage disease in the brain: Mutations of the β-mannosidase gene identified in autosomal dominant nystagmus. Genet Med 17: 971-979, 2015.
22. Cai T, Yang L, Cai W, Guo S, Yu P, Li J, Hu X, Yan M, Shao Q, Jin Y, *et al*: Dysplastic spondylolysis is caused by mutations in the diastrophic dysplasia sulfate transporter gene. Proc Natl Acad Sci USA 112: 8064-8069, 2015.