

test sites in screening for DSP. This will maximize the probability of obtaining accurate and comparable results at different assessment times.

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Mutations of the slow muscle α -tropomyosin gene, *TPM3*, are a rare cause of nemaline myopathy

Abstract—The α -tropomyosin-3 (*TPM3*) gene was screened in 40 unrelated patients with nemaline myopathy (NM). A single compound heterozygous patient was identified carrying one mutation that converts the stop codon to a serine and a second splicing mutation that is predicted to prevent inclusion of skeletal muscle exon IX. *TPM3* mutations are a rare cause of NM, probably accounting for less than 3% of cases. The severity of cases with *TPM3* mutations may vary from severe infantile to late childhood onset, slowly progressive forms.

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Nemaline myopathy (NM) is a clinically and genetically heterogeneous disorder characterized by nemaline rods and skeletal muscle weakness that ranges in severity from a neonatally life-threatening disorder to mild muscle weakness of adulthood.^{1,2} Nema-

line rods appear as abnormalities of the Z lines associated with disruption of thin filament organization suggesting that they may reflect primary abnormalities of thin filament proteins. The *TPM3* gene encodes the slow (type 1) fiber-specific isoform of skeletal muscle α -tropomyosin. A *TPM3* missense mutation, Met9Arg, was identified in an Australian family with autosomal dominant transmission of a late childhood onset, slowly progressive, form of NM.³ Follow-up studies of the *TPM3* gene in 76 NM cases have identified only 1 additional mutation, a homozygous nonsense mutation in a severe infantile case of NM.⁴ In contrast, many cases result from mutations in the nebulin or actin genes, whereas troponin T (*TNNT1*) and β -tropomyosin (*TPM2*) gene mutations are rare causes of NM.^{1,5} We report the identification of the 3rd and the 4th distinct *TPM3* mutations in an intermediate severity case of NM, demonstrating further allelic and clinical heterogeneity among patients with *TPM3* abnormalities.

Methods. Patients. Patients with NM from North American neuromuscular clinics were enrolled after institutional review board-approved informed consent. Forty unrelated probands included 7 with the severe congenital

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form of nemaline myopathy, 15 with intermediate presentations (did not achieve ambulation by 2 years or became nonambulant by age 11 years), 15 with congenital onset of myopathy and slowly or nonprogressive courses, and 3 cases of childhood onset NM.^{1,2} All cases were either sporadic or consistent with autosomal recessive inheritance with the exception of four families with dominant inheritance. Patient 13-2 was a sporadic case who was hypotonic at birth and walked at 17 months, but became wheelchair bound at age 6 years. Muscle biopsy at age 5 years revealed subsarcolemmal and intracytoplasmic nemaline rods in type 1 fibers, type 1 fiber predominance, central nuclei, and increased endomysial connective tissue (figure 1; additional clinical details are available in the Methods section of the supplementary data; go to www.neurology.org).

Molecular genetics. Detailed methodology is available as part of the supplementary data (additional material related to this article can be found on the *Neurology* Web site; go to www.neurology.org).

Results. *TPM3* mutation screening was normal in 39 probands; however, single-strand conformation polymorphism analysis of exon IXsk revealed an aberrant conformer in one patient, 13-2, and his unaffected father. Sequence analysis revealed that this conformer contained an A to C mutation at nucleotide 915 of the skeletal muscle mRNA eliminating the normal skeletal muscle-specific translational stop signal, replacing it with a serine residue (TCA) at codon 285 (figure 2). Consequently, the mutated protein includes an additional 57 amino acids (figure 3B). Direct sequencing of the patient's genomic PCR products confirmed that he was heterozygous for this mutation and identified a second heterozygous mutation, an AG to AA at the acceptor splice site of the same exon (see figure 2A). This change was evident in genomic DNA of the unaffected mother, but not in the father. Neither mutation was found in 109 unaffected control individuals.

To determine the effects of the presumed maternal splice site mutation, rtPCR was performed using a reverse primer in exon IXsk, sk986L. As expected if the maternal exon IXsk is not properly spliced, rtPCR products from the proband's muscle contained only the mutant paternal transcript although the father's muscle contained both mutant and wild type transcripts (see figure 2B, C). In contrast, rtPCR analysis of the proband's mRNA using an exon IXsk forward primer and a nonmuscle exon VIIIIm reverse primer did reveal an abnormal splice product in which exon VIIIIm skipped exon IXsk and was instead spliced to exon VIIIIm (see figure 2B, D). Remarkably, because of a frame shift, this aberrant splice product recreates a normal stop codon in the same location as encoded by exon IXsk. However, the clinical phenotype of Patient 13-2 suggests that this aberrant splicing does not occur at sufficient frequency to prevent rod formation and clinical weakness.

Western blotting for tropomyosins in the muscle of Patient 13-2 revealed the presence of a novel reactive protein approximately 6 kD larger than normal (~34 kD) α -tropomyosin (see figure 3A). Interestingly, the ~36 kD band for β -tropomyosin, which migrates above that for α -tropomyosin and is also detected by the CH1 antibody,^{6,7} appeared significantly reduced relative to α -tropomyosin levels (reflecting both *TPM1* and *TPM3* gene products).

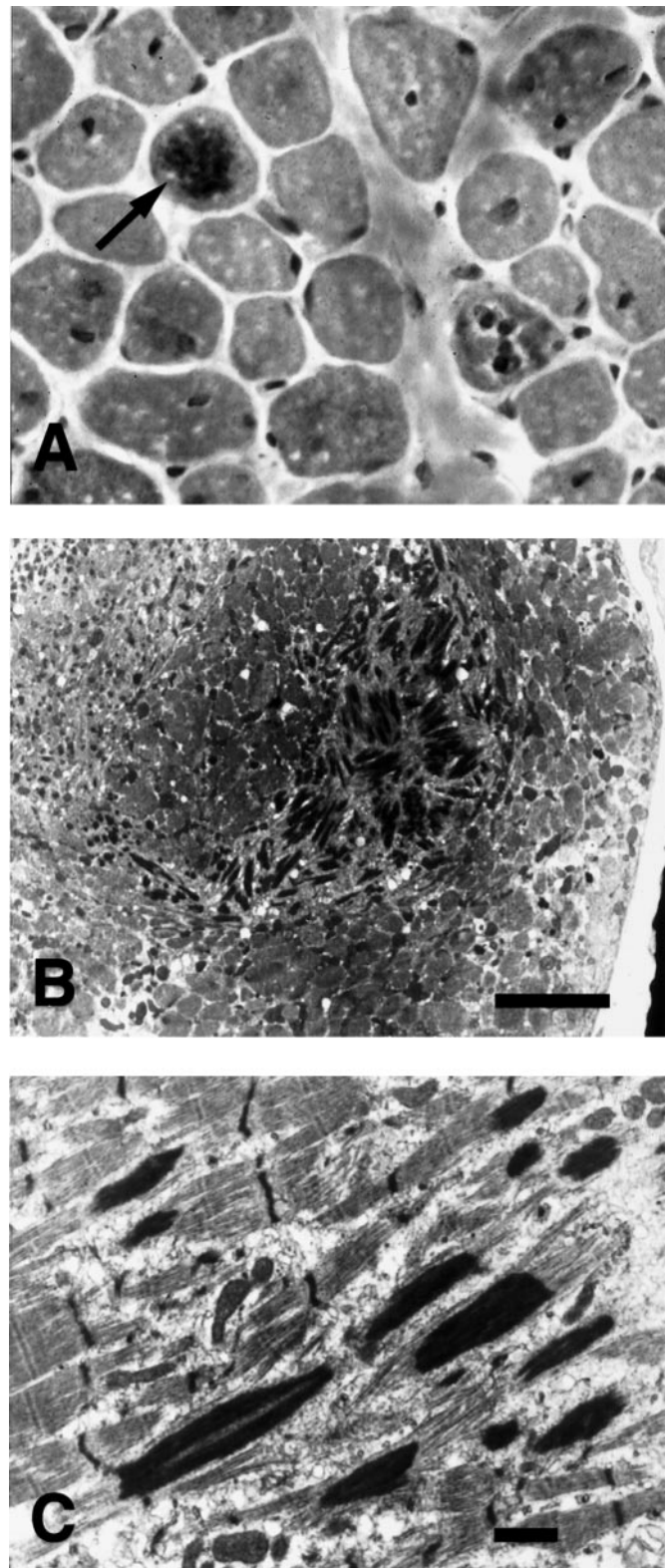


Figure 1. Pathologic findings in quadriceps muscle from Patient 13-2. (A) Modified Gomori trichrome staining illustrating intracytoplasmic rod aggregation (arrow), occasional central nuclei, and increased endomysial fibrosis. Original magnification 200X. (B) Electron micrograph revealing central aggregation of rod bodies. Scale bar = 5 μ m. (C) Electron micrograph showing typical nemaline rods emanating from Z lines and disrupting the normal sarcomeric architecture. Scale bar = 1 μ m.

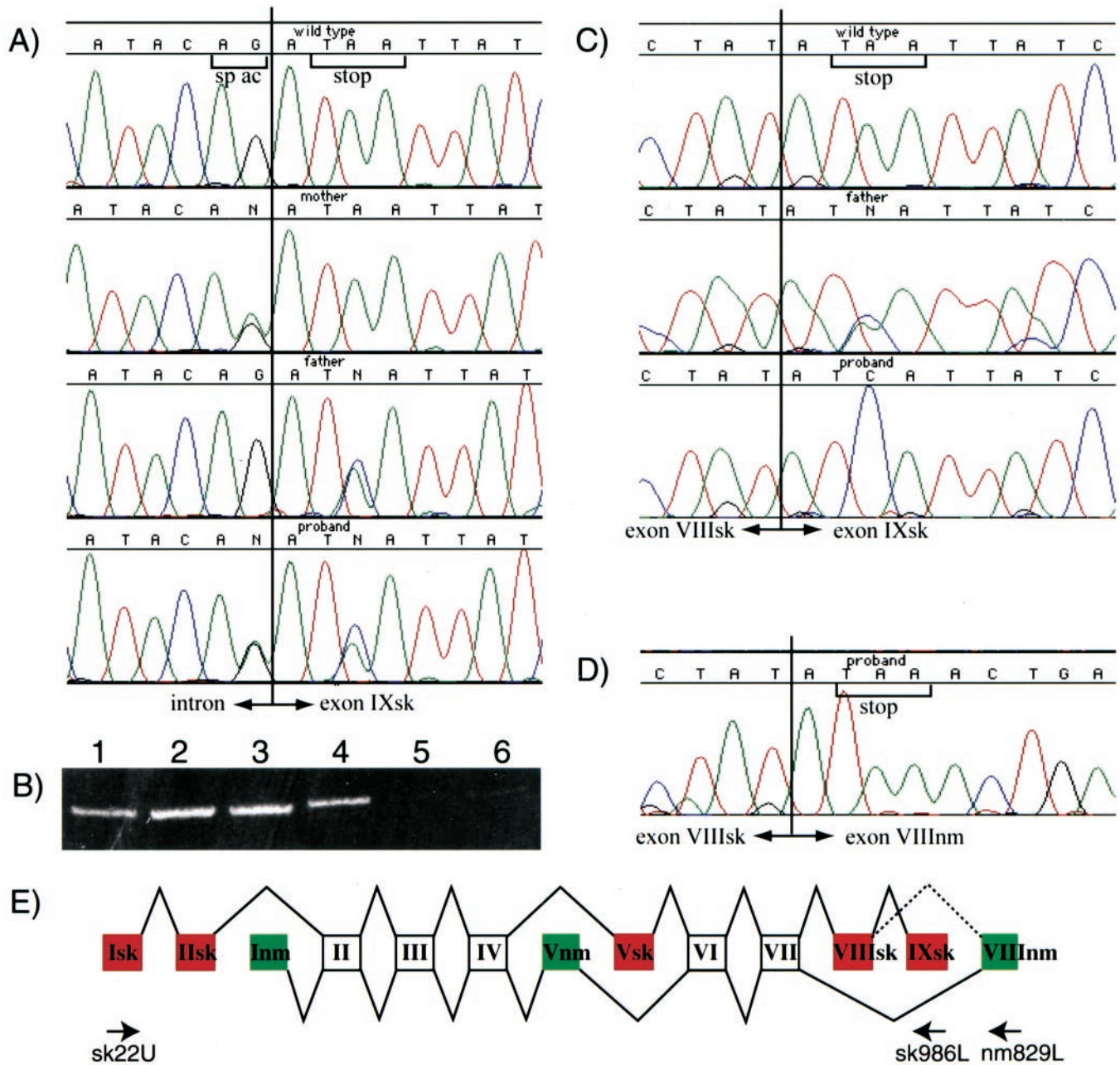


Figure 2. TPM3 mutation analysis. Intron–exon and exon–exon boundaries are indicated by a vertical line and annotated below each chromatogram. (A) DNA sequence analysis of genomic PCR products containing TPM3-exon IXsk and adjacent intronic sequences illustrating wild type (top row), maternal splice acceptor site AG to AA mutation (second row), paternal stop codon A915C mutation (third row), and double heterozygosity for these in the proband (bottom row). (B) rtPCR analysis of normally spliced skeletal muscle transcripts (using primers sk22U and sk986L) (lanes 1–3) and abnormally spliced transcripts (using primers sk22U and nm829L) (lanes 4–6) in Patient 13-2 (lanes 1,4), his father (lanes 2,5), and a control subject (lane 3,6). (C) Sequence analysis of the exon VIIIsk-IXsk junction in rtPCR products from B, lanes 1–3, from control (top), proband’s father’s (middle), and the proband’s (bottom) skeletal muscle. Although the proband’s father expresses both mutant and wild type alleles at similar levels, only the mutant paternal allele is represented in the proband when this combination of primers is used. (D) Sequence analysis of the proband’s skeletal muscle mRNA using sk22U and nm829L primers (B, lane 4) illustrating the aberrant splice product containing exon VIIIsk spliced to exon VIIIInm. Notably, because of a frameshift this introduces an in-frame stop codon (TAA) at precisely the same location as that of exon IXsk (indicated). (E) Schematic diagram of the TPM3 gene illustrating tissue-specific exons [red = muscle (“sk”), green = nonmuscle (“nm”)] with the normal skeletal muscle and nonmuscle splicing patterns indicated above and below. The aberrant exon VIIIsk-VIIIInm splice product is indicated by dashed line. Approximate locations of PCR primers used in the rtPCR analysis are shown below.

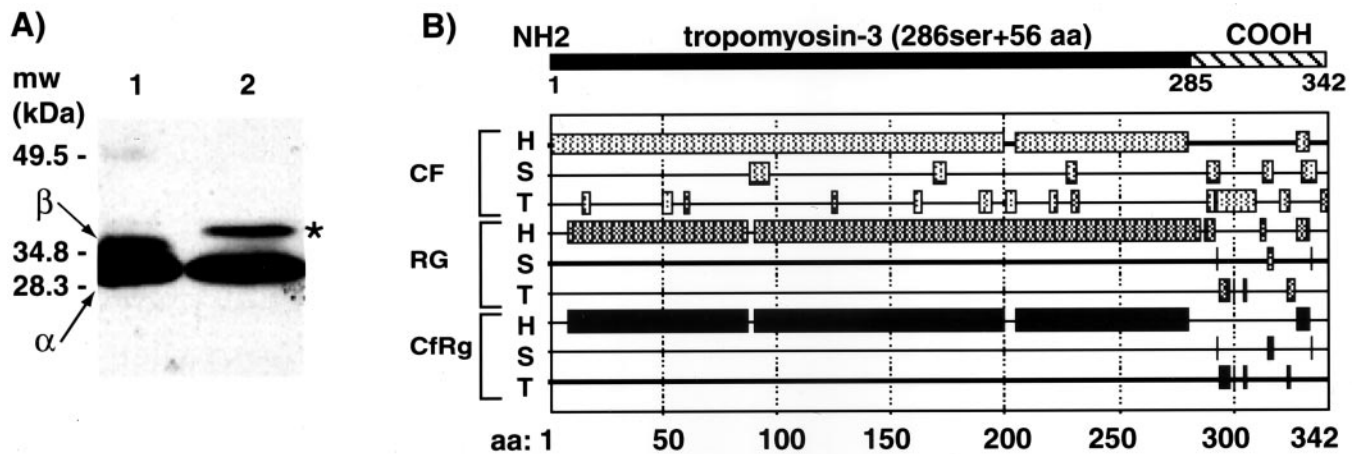


Figure 3. (A) Western blot analysis of skeletal muscle tropomyosins from normal control (deltoid) muscle (lane 1) and quadriceps muscle from Patient 13-2 (lane 2) using monoclonal anti-sarcomeric tropomyosin antibody clone CH1. Two bands of apparent mw 34 kD (α -tropomyosin) and 36 kD (β -tropomyosin) are evident in the control muscle.⁷ A novel band of approximately 40 kD is evident in lane 2 (asterisk). (B) Secondary structure predictions for the mutant α -tropomyosin 3 protein shown schematically at top. Filled portion includes residues 1–284 of the wild type skeletal isoform. Hatched area indicates the additional 57 amino acids predicted at the carboxy terminus of the abnormal protein. Shown are predicted regions of alpha-helix (H), beta-pleated sheet (S), and turn (T), based on Chou-Fasman (CF), Robson-Garnier (RG), and combined (CfRg) prediction algorithms. Note extensive α -helical structure of residues 1–284 that contribute to coiled-coil motifs in the native protein.

Discussion. This study confirms the role of *TPM3* mutations in rare cases of both autosomal dominant and recessive forms of NM and demonstrates clinical heterogeneity associated with *TPM3* abnormalities. Although it is impossible to draw firm conclusions based on only three known instances of *TPM3* mutation, it may be that the relative degrees of severity relate to the nature of the underlying mutations. The mildest cases are all from the single family with the missense mutation, Met9Arg.³ In contrast, the most profoundly weak patient was homozygous for a nonsense mutation at codon 31 that prevented production of stable and functional protein.⁴ The intermediate course of Patient 13-2 may be caused by his compound heterozygosity for the exon IXsk acceptor splice site mutation and the termination codon mutation leading to production of a partially functional, enlarged protein product. Because tropomyosins form heterodimers between different isoforms (including α/β forms),⁸ one might predict that the *285Ser mutation would act in a trans-dominant fashion. However, the lack of pathologic changes in the muscle or clinical disease in the patient's father, Subject 13-1, demonstrates that this is not the case, perhaps suggesting that the extensive disruption of α -helical coiled-coil structure at the carboxy terminus of the *285Ser mutant protein prevents dimerization.

Expression of *TPM3* is limited to type 1 fibers,⁸ as was rod formation in Patient 13-2. Nevertheless, his biopsy exhibited secondary type 1 fiber predominance, likely reflecting a dynamic pathologic process associated with the patient's loss of ambulation at age 5 years. The apparent reduction in levels of β -tropomyosin appears paradoxical in light of the belief that type 1 fibers contain relatively higher ratios

of β - to α -tropomyosin than type 2 fibers.⁸ However, Salviati et al.,⁷ in protein analyses of single human skeletal muscle fibers, have identified two populations of type 1 fibers, one of which expressed both α -tropomyosin (e.g., *TPM1* and *TPM3*) isoforms and very little β -tropomyosin.⁷ The relative lack of β -tropomyosin in the context of fiber type 1 predominance suggests that muscle in Patient 13-2 preferentially contains this subset of type 1 fibers. Potential functional or clinical consequences of this observation are presently unknown.

The last nine amino acid residues of skeletal muscle α -tropomyosin-3 determine the binding affinity of unacetylated tropomyosin for actin.⁹ Furthermore, intact exon IXsk is required for the troponin complex to promote the high affinity of tropomyosin for skeletal actin. Thus, alteration of carboxy-terminal sequences, such as the addition of a non- α -helical tail, might be predicted to considerably alter the interactions between tropomyosin and actin in thin filaments, possibly accounting for the nemaline body formation and muscle weakness in our patient. Alternatively, by analogy to Tan et al.'s case,⁴ rod formation may simply result from perturbation of the ratio of functional tropomyosin to other thin filament components in type 1 fibers.

Both Patient 13-2 and the previously reported recessive case⁴ exhibited a more severe clinical progression than many of those with nebulin-associated NM, whereas many patients with actin mutations have had severe nemaline myopathy.¹ However, additional patients with NM with *TPM3*, nebulin, and actin mutations will need to be characterized before any prognostic predictions can be made. Although the proportion of NM cases caused by *TPM3* mutations

appears to be low (e.g., 3 of 116 studied families = 2.6%),^{3,4} the relatively small size of the *TPM3* gene and transcript allows for rapid mutation testing, which is now becoming an important part of the molecular genetic workup for NM.

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Asymptomatic cores and paracrystalline mitochondrial inclusions in CADASIL

Abstract—Three siblings with genetically assessed cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL) with core-like lesions and mitochondrial abnormalities in muscles are described. Involvement of the Ryanodine receptor 1 gene was excluded. In the current cases, the relation between molecular genetic lesion and muscle fiber abnormalities remains to be determined, but the Notch3 gene may influence mitochondrial metabolism.

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To date, deposition of granular osmiophilic material in the smooth muscle cells of precapillary arterioles of the skin, muscle, peripheral nerve, heart, and kidney has been regarded as the only extracerebral involvement in cerebral autosomal dominant arteriopathy with subcortical infarcts and leukoencephalopathy (CADASIL).^{1,2} These lesions do not have clinical manifestations. Clinical signs are confined to the CNS even in advanced stages. Here, we describe distinctive but asymptomatic skeletal muscle involvement in three siblings with CADASIL.

Methods and results. Clinical and laboratory findings for this family have been reported previously.³

For all three patients, brain MRI showed many bilateral and almost symmetric areas of hyperdensity of the cerebral white matter on T2-weighted images. None of the three patients had EMG changes or elevated serum creatine kinase levels.

Patient 1. A 52-year-old man had right facial paresthesia and headache from the age of 37 years. Similar episodes had since occurred about once a year. From the age of 40 to 41 years, he had progressive mental deterioration. Occasional episodes of transient weakness on the right side and aphasia had occurred in recent years. Neurologic examination showed mild impairment of cognitive function (IQ, 80) and tendon reflexes accentuated on the right side.

Patient 2. A 58-year-old woman had frequent episodes of headache with dizziness from approximately age 40 years. At 52 years old, she presented with severe headache and disorientation. In recent years, episodes of amnesia and slight behavior disturbances also have been noticed. Neurologic examination showed only slight mental deterioration.

Patient 3. A 60-year-old woman had occasional episodes of headache, visual impairment, speech disturbance,

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