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CGG/CCG Repeat Expansions in *LOC642361/NUTM2B-AS1* in Thai Patients With Oculopharyngodistal Myopathy

Sunsanee Pongpakdee, MD, Metha Apiwattanakul, MD, Thanes Termglinchan, MD, Rawiphan Witoonpanich, MD, Charungthai Dejthevaporn, MD, Theeraphong Lee, MD, Supika Wansophonkul, MD, Ai Yamanaka, MD, Shunsuke Funaguma, PhD, Aritoshi Iida, PhD, and Ichizo Nishino, MD, PhD

Correspondence
Dr. Nishino
nishino@ncnp.go.jp

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Abstract

Objectives

This study characterizes oculopharyngodistal myopathy in 4 Thai patients from 3 families with CGG/CCG repeat expansion in *LOC642361/NUTM2B-AS1*.

Methods

Repeat-primed PCR analyzed CGG/CCG repeat size in *LOC642361/NUTM2B-AS1* in 4 Thai patients suspected of oculopharyngodistal myopathy (OPDM). Clinical records were reviewed for clinicopathologic features.

Results

All patients exhibited strong somatic instabilities of the expanded CGG/CCG repeats, primarily manifesting as oculopharyngeal weakness. Patient 1 had mild finger extensor and intrinsic hand muscle weakness, and although patient 2 lacked limb weakness, both siblings showed electrophysiologic evidence of distal myopathy, indicative of OPDM. Patient 3, the daughter of a sibling with OPDM reported in 2004, lacked limb weakness or leukoencephalopathy on brain MRI. Patient 4, initially misdiagnosed with refractory myasthenia gravis, had generalized muscle weakness.

Discussion

While initially characterized as oculopharyngeal myopathy with leukoencephalopathy (OPML) in a Japanese family, our study suggests a stronger association between CGG/CCG expansion in *LOC642361/NUTM2B-AS1* and oculopharyngodistal myopathy (OPDM) rather than OPML. The variable presence or absence of leukoencephalopathy further supports OPDM as the predominant clinical manifestation linked to CGG/CCG expansion in *LOC642361/NUTM2B-AS1*.

Introduction

Oculopharyngodistal myopathy (OPDM) is a rare genetic muscle disease caused by CGG repeat expansion in genes, namely *LRP12*[1], *GIPC1*[2], *NOTCH2NLC*[3,4], *RILPL1*,[5] and *ABCD3*.[6] Common features include ptosis, ophthalmoparesis, dysarthria, dysphagia, and distal weakness. In 2019, *LRP12* was reported as the first causative gene of OPDM and *LOC642361/NUTM2B-AS1* was reported in 1 family with oculopharyngeal myopathy with leukoencephalopathy

From the Department of Medicine (S.P.), Bhumibol Adulyadej Hospital; Department of Neurology (M.A., T.T.), Neurological Institute of Thailand; Department of Medicine (R.W., C.D.), Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok; Department of Medicine (T.L.), HRH Princess Sirindhorn Hospital, Rayong; Department of Radiology (S.W.), Bhumibol Adulyadej Hospital, Bangkok, Thailand; Department of Neuromuscular Research (A.Y., I.N.), National Institute of Neuroscience, National Center of Neurology and Psychiatry (NCNP); and Department of Clinical Genome Analysis (S.F., A.I., I.N.), Medical Genome Center, NCNP, Tokyo, Japan.

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(OPML).[1] We found 4 Thai patients with oculopharyngeal involvement, who tested negative for *PABPN1, LRP12, GIPC1, NOTCH2NLC,* and *RILPL1*. These patients differed from those with typical OPML, showing an oculopharyngeal onset and evidence of distal myopathy, with or without minimal white matter change on brain MRI.

Methods

Genetic Analysis

Genomic DNA was extracted from peripheral blood of the patients and their families using standard protocols. We sequenced patient 1 using Nanopore PromethION with the R9.4.1 flow cell (Oxford Nanopore Technologies) according to the manufacturer's instructions. The Nanopore long reads were aligned to the human genome (GRCh38/hg38) using LAST and then screened for disease-associated repeat expansion with tandem-genotypes algorithm.[7] We performed repeat-primed PCR (RP-PCR) and fragment analysis as described in earlier studies, with minor adjustments.[1,2] For the fragment analysis, the PCR primer set was as follows: LOC642361_F2: 5′-ACGCGCTGCGCG-GACGGGA-3′, LOC642361_R_FAM: 5'-[FAM]CGCTA-GAAGGAGTGTGGTCCACC-3'. The PCR product length was 229 bp containing $(GGC)_7$ repeats.

Nanopore Methylation Calls

We constructed a custom human genome reference, in which we inserted A_{20} followed by $((CGG)_{13} + A_{11})_{53}$ between chr10:79824000 and 79824001 in the hg38 human genome as the reference sequence of an expanded CGG/CCG repeat. We analyzed the methylation status of CpG sites within the $(CGG)_n$ repeat sequence at every 50-bp interval. We aligned FASTQ files of patient 1 to the custom reference genome using minimap2 (v2.28-r1209)[7] and sorted with samtools (v1.15.1).[8] We called the methylation status of CpGs within an expanded CGG/CCG repeat in *LOC642361/NUTM2B-AS1* using Nanopolish (v0.14.0)[9] and visualized the result using methylartist (v1.3.0).[10]

Results

Patient 1 (Family 1, III-4)

A 37-year-old Thai man (Figure 1A) presented with bilateral ptosis since age 25. He observed voice changes at 32 years, followed by difficulty swallowing and lifting heavy objects. He complained of postmeal bloating and urinary urgency but no incontinence. Despite occasional knee weakness, he could still run up to 5 km. His family history included similar symptoms in his elder brother (Figure 1B), grandmother, and father (Figure 1C).

Physical examination showed a slender build and a high-arched palate. He had bilateral ptosis, slow eyelid movements, left eye hypertropia, and asymmetrical ophthalmoplegia. Other findings included nasal voice, facial diplegia, temporalis muscle atrophy, restricted mouth opening, and mild weakness of tongue, neck flexors, intrinsic hand, and bilateral finger extensor muscles. His muscle tone was normal, with reduced reflexes and no Babinski sign. Cardiovascular, respiratory, and abdominal examinations were unremarkable. Eye and hearing tests were normal.

Cognitive assessments (MOCA and MMSE-Thai) were normal. Serum creatine kinase (CK) was 178 U/L (normal: 24–190). EKG and echocardiogram were normal. Brain MRI revealed symmetrical T2/FLAIR signal hyperintensities along the corticospinal tracts, thalami, and internal capsules while sparing the subcortical white matter. Muscle pathology showed some fibers with rimmed vacuoles, in addition to fiber size variation.

Patient 2 (Family 1, III-1)

The older brother of patient 1, aged 39 years, reported bilateral ptosis persisting for 4 years, accompanied by gradual voice changes and difficulty swallowing solid and liquid foods. He occasionally experienced coughing and nasal regurgitation during meals, along with postmeal bloating. He denied double vision and limb weakness, but a reduced mouth opening was noted. Examination revealed nasal voice, bilateral ptosis, slow eyelid movements, asymmetrical ophthalmoplegia, and facial diplegia. Neck flexor weakness was evident, but limb muscles were unaffected. Reflexes were diminished, and no Babinski sign was noted. Uvula, gag reflex, mastication, and tongue functioned normally.

Serum CK was elevated at 211 U/L. Brain MRI showed symmetrical T2/FLAIR signal hyperintensities only in the corticospinal tract on both sides. His symptoms resembled those of patient 1 but less severe, although brain MRI findings were more prominent (Figure 1D). Cognitive assessments (MOCA and MMSE-Thai) were in normal range.

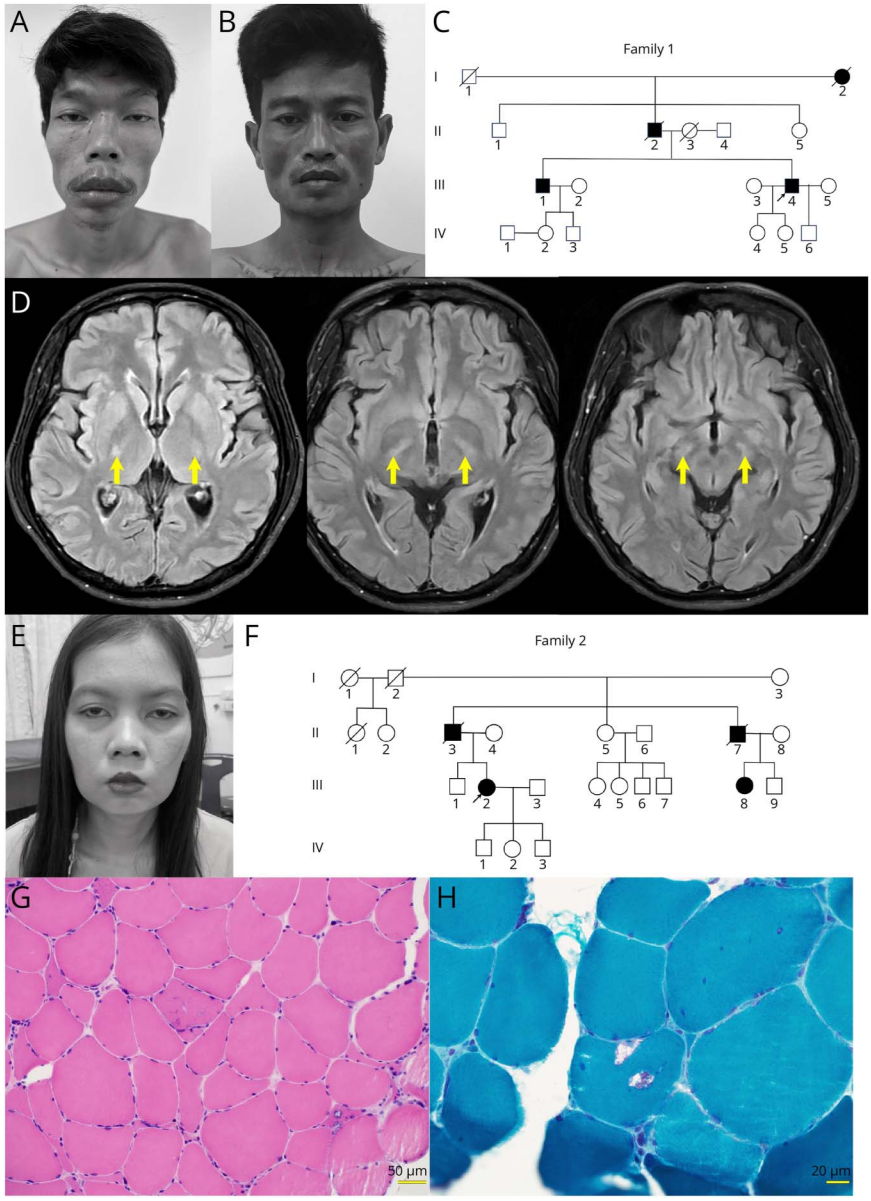
Electrophysiologic studies for both patients revealed normal nerve conduction study and excluded large fiber polyneuropathy. Needle EMG showed irritative myopathic changes predominantly in the distal leg muscles. Abnormal single-fiber EMG in the orbicularis oculi was observed in both cases.

Patient 3 (Family 2, III-2)

A 35-year-old woman (Figure 1E) presented with bilateral ptosis over 5 years, with 3 years of voice changes and 2 years of mild dysphagia, without limb weakness. Examination showed a nasal voice, bilateral ptosis, ophthalmoparesis, weakness in the orbicularis oculi, and mild neck extensor weakness, without limb weakness. All deep tendon reflexes were absent, and sensory examination was normal. Serum CK was elevated to 1,339 U/L. EKG and brain MRI were normal.

Her cousin (III-8) had similar features but was unavailable for examination. Her father (II-3) and his younger brother (II-7) experienced bilateral, slowly progressive ptosis starting at ages 35 and 33, respectively, followed by abnormal voice, slight

Figure 1 Photos of Patients, Pedigrees, Brain MRI, and Muscle Pathology



(A) Patient 1, (B) patient 2, (C) pedigree of family 1, (D) brain MRI of patient 2 showing faint symmetrical T2/FLAIR hyperintense changes bilaterally at the ventral cerebral peduncles, ventrolateral thalami, and posterior limb of internal capsules, (E) patient 3, (F) pedigree of family 2, and (G) hematoxylin and eosin stain and (H) modified Gomori trichrome stain of muscle pathology of patient 4 showing marked variation in fiber size, no apparent necrotic fibers, a few regenerating fibers, and several fibers with rimmed vacuoles.

difficulty swallowing, and, later, wasting of the forearm and hand muscles (Figure 1F). Muscle biopsy revealed a few fibers with rimmed vacuoles. Genetic analysis excluded GCN repeat expansion in *PABPN1*, leading to the clinical diagnosis of oculopharyngodistal myopathy (OPDM), as previously reported.[11]

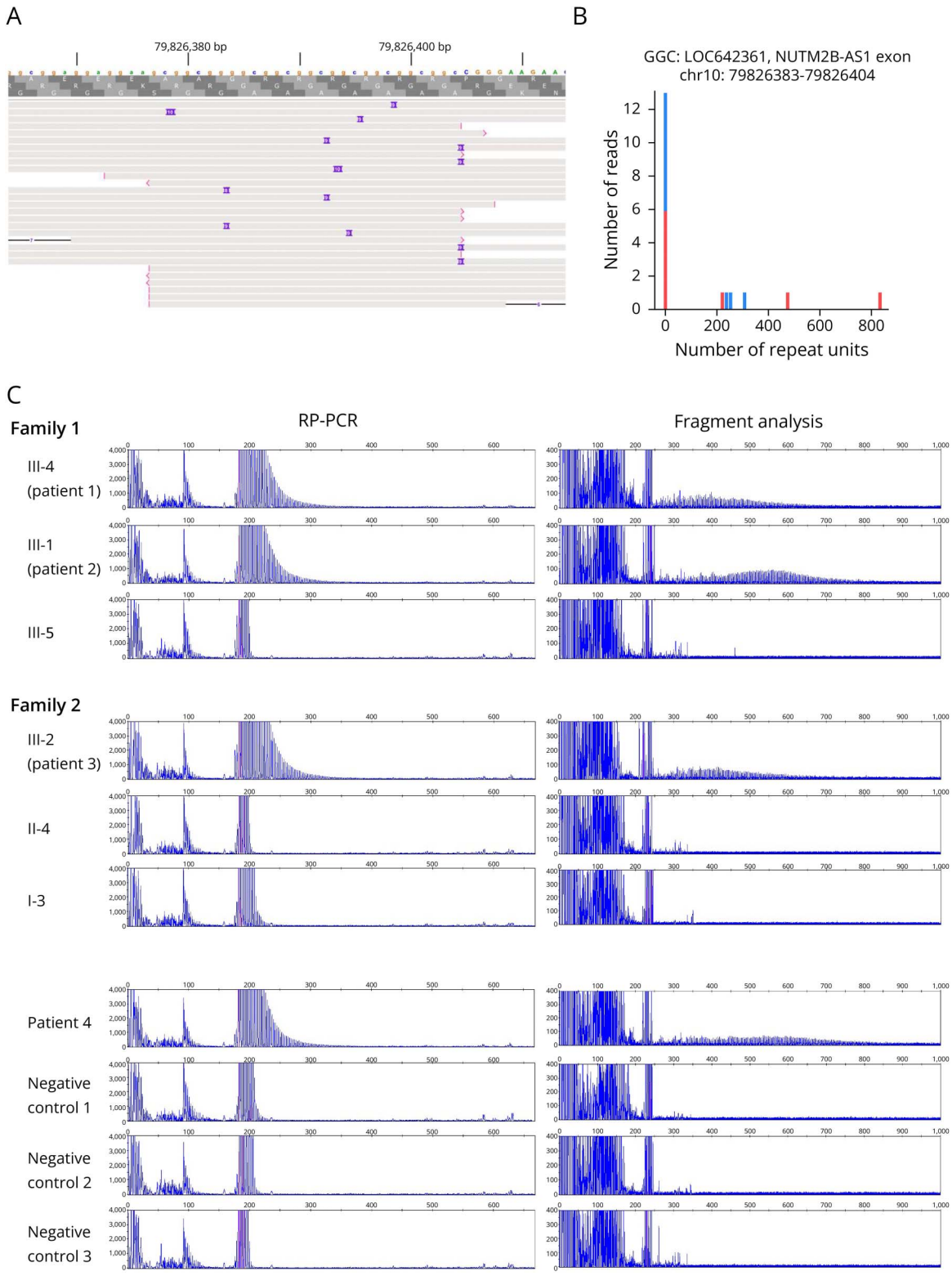
Patient 4

A 47-year-old woman presented with a 6-year history of bilateral ptosis, total ophthalmoplegia, nasal voice, and difficulty walking upstairs for 1 year. Physical examination revealed a high-arched palate, tongue atrophy, bilateral hip flexor weakness, and generalized hyporeflexia. She had been unsuccessfully treated with corticosteroids and pyridostigmine for suspected myasthenia gravis. No family members had a similar illness. Serum CK levels were normal. MRI of the thigh and leg revealed a pronounced alteration in proximal muscles, surpassing the changes observed in distal muscles. Muscle pathology showed some fibers with rimmed vacuoles (Figure 1, G and H).

Genetic Analysis

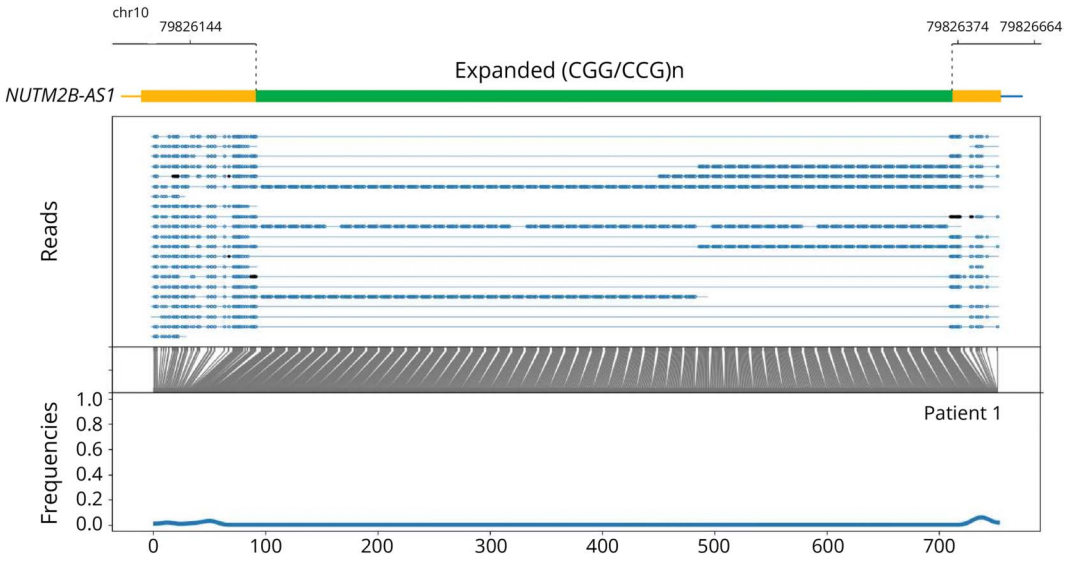
To diagnose 4 patients genetically, genetic analyses including RP-PCR for 4 OPDM causative genes (*LRP12*, *GIPC1*, *NOTCH2NLC*, and *RILPL1*) and the original targeted gene panel were performed but were found to be negative. We then screened the pathogenic variant in one patient (patient 1) by Nanopore long-read whole-genome sequencing and identified a CGG/CCG repeat expansion in *LOC642361/NUTM2B-AS1* exceeding 20 times the count found in the normal allele

Figure 2 Genetic Analysis of *LOC642361/NUTM2B-AS* in Thai Patients With OPDM



(A) Long reads containing CGG/CCG repeat expansions in *LOC642361/NUTM2B-AS1* were aligned to the Homo sapiens (human) genome assembly GRCh38/hg38 with the integrative Genomics Viewer coupled with LAST. (B) More than 200 repeat units of CGG/CCG in patient 1 are shown. The x-axis indicates the change in the repeat number relative to GRCh38/hg38. The y-axis indicates the number of reads. Red and blue bars show forward and reverse strand reads, respectively. (C) RP-PCR (left panel) and fragment analysis (right panel) showing CGG/CCG repeat expansions in patients with OPDM.

Figure 3 Detection of CpG Methylation State of an Expanded CGG/GGC Repeat in *LOC642361/NUTM2B-AS1*



The panels show the following information from top to bottom: the genome region on chr10 (GRCh38/hg38), *LOC642361/NUTM2B-AS1* (exon 1 as an orange box, intron as an orange line, an expanded CGG/GGC repeat as a green box, and an regulatory region as a blue line), CpG sites marked as open or closed circles, and a smoothed plot of the methylation profile. The x-axis of this smoothed plot indicates methylation bins used in the smoothed methylation profile plot. The y-axis indicates methylation frequencies in Nanopore raw reads in patient 1.

(Figure 2, A and B). These repeat expansions in patient 1 and his affected and unaffected members were validated using RP-PCR and fragment analysis. In addition, RP-PCR and fragment analysis revealed the *LOC642361/NUTM2B-AS1* repeat expansions in 2 unrelated Thai families with OPDM (Figure 2C). CGG/CCG repeat size was highly variable in each patient and was estimated to more than 100 repeats, whereas CGG/CCG repeat sizes in negative controls ranged from 9 to 13 (Figure 2C). We also analyzed methylation status of expanded CGG/CCG repeat sequence and its flanking regions in the *LOC642361/NUTM2B-AS1* in patient 1. The CpG sites in the expanded CGG/CCG repeat were hypomethylated (Figure 3).

Discussion

In this study, CGG/CCG repeat expansions in *LOC642361/NUTM2B-AS1* were identified in 4 Thai patients from 3 unrelated OPDM families. *LOC642361/NUTM2B-AS1* are bidirectionally transcribed, long noncoding RNAs. A CGG/CCG repeat were in overlapping noncoding exons in both LOC642361 and NUTM2B-AS1 transcripts. Strong somatic instability of CGG/CCG repeats was observed in genomic DNA from the patient's peripheral blood leukocytes by southern blotting. Fragment analysis also showed that 4 patients had highly unstable CGG/CCG repeat expansions that varied in length in the somatic cells. In line with the report,[12] methylation of CpGs of the expanded CGG/CCG repeat in the *LOC642361/NUTM2B-AS1* was not observed in patient 1.

OPDM was initially documented in Japan in 1977.[13] The causative genes were only recently identified, primarily in Asian populations, including *LRP12*[1], *GIPC1*[2], *NOTCH2NLC*[3,4], *and RILPL1*.[5] However, *ABCD3*[6] has recently emerged as a causative gene in European cases. In 2019, *LOC642361/NUTM2B-AS1* were linked to oculopharyngeal myopathy with leukoencephalopathy (OPML).[1] While patients with OPML exhibited widespread weakness, in addition to cardiac, respiratory, and gastrointestinal involvement, our patients primarily presented with oculopharyngeal weakness and 2 showed evidences of distal myopathy, suggesting that the major phenotype may well be OPDM. This perspective is reinforced by 2 recent reports describing altogether 14 Chinese patients with OPDM from 2 recent articles with CGG/GCC repeat expansion in *LOC642361/NUTM2B-AS1*.[12,14] Long-term follow-up is essential for our patients who currently do not show limb muscle weakness because distal limb weakness may appear over time. Patients with OPML have been reported to exhibit extensive white matter changes on brain MRI. By contrast, 2 of our patients exhibited T2/FLAIR signal hyperintensities in the corticospinal tracts while sparing the subcortical white matter. This suggests that leukoencephalopathy may not always develop in this disease but, if it does, it may start in the corticospinal tract.

In conclusion, our findings, along with the recent Chinese studies, suggest that *LOC642361/NUTM2B-AS1* variation primarily manifests as OPDM, with or without leukoencephalopathy, rather than OPML.

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Disclosure

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Appendix Authors

Name	Location	Contribution
Sunsanee Pongpakdee, MD	Department of Medicine, Bhumibol Adulyadej Hospital, Bangkok, Thailand	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; analysis or interpretation of data
Metha Apiwattanakul, MD	Department of Neurology, Neurological Institute of Thailand, Bangkok, Thailand	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data
Thanes Termglinchan, MD	Department of Neurology, Neurological Institute of Thailand, Bangkok, Thailand	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data
Rawiphan Witoonpanich, MD	Department of Medicine, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data
Charungthai Dejthevaporn, MD	Department of Medicine, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand	Major role in the acquisition of data; analysis or interpretation of data
Theeraphong Lee, MD	Department of Medicine, HRH Princess Sirindhorn Hospital, Rayong, Thailand	Major role in the acquisition of data
Supika Wansophonkul, MD	Department of Radiology, Bhumibol Adulyadej Hospital, Bangkok, Thailand	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; analysis or interpretation of data
Ai Yamanaka, MD	Department of Neuromuscular Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry (NCNP), Tokyo, Japan	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; analysis or interpretation of data
Shunsuke Funaguma, PhD	Department of Clinical Genome Analysis, Medical Genome Center, NCNP, Tokyo, Japan	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; analysis or interpretation of data
Aritoshi Iida, PhD	Department of Clinical Genome Analysis, Medical Genome Center, NCNP, Tokyo, Japan	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; analysis or interpretation of data
Ichizo Nishino, MD, PhD	Department of Neuromuscular Research, National Institute of Neuroscience, National Center of Neurology and Psychiatry (NCNP); Department of Clinical Genome Analysis, Medical Genome Center, NCNP, Tokyo, Japan	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design; analysis or interpretation of data

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