REVIEW OPEN ACCESS

Review of Phenotypic Heterogeneity of Neuronal Intranuclear Inclusion Disease and *NOTCH2NLC*-Related GGC Repeat Expansion Disorders

Tao Zhang, MD, Lei Bao, PhD, and Hao Chen, MD

Correspondence
Dr. Bao
baolei56@gmail.com

Neurol Genet 2024;10:e200132. doi:10.1212/NXG.0000000000200132

Abstract

Neuronal intranuclear inclusion disease (NIID) is an underdiagnosed neurodegenerative disorder caused by pathogenic GGC expansions in *NOTCH2NLC*. However, an increasing number of reports of *NOTCH2NLC* GGC expansions in patients with Alzheimer disease, essential tremor, Parkinson disease, amyotrophic lateral sclerosis, and oculopharyngodistal myopathy have led to the proposal of a new concept known as *NOTCH2NLC*-related GGC repeat expansion disorders (NREDs). The majority of studies have mainly focused on screening for *NOTCH2NLC* GGC repeat variation in populations previously diagnosed with the associated disease, subsequently presenting it as a novel causative gene for the condition. These studies appear to be clinically relevant but do have their limitations because they may incorrectly regard the lack of MRI abnormalities as an exclusion criterion for NIID or overlook concomitant clinical presentations not typically observed in the associated diseases. Besides, in many instances within these reports, patients lack pathologic evidence or undergo long-term follow-up to conclusively rule out NIID. In this review, we will systematically review the research on *NOTCH2NLC* 5′ untranslated region GGC repeat expansions and their association with related neurologic disorders, explaining the limitations of the relevant reports. Furthermore, we will integrate subsequent studies to further demonstrate that these patients actually experienced distinct clinical phenotypes of NIID.

Introduction

Neuronal intranuclear inclusion disease (NIID) is indeed an uncommon hereditary neurodegenerative condition. It arises because of GGC repeat expansions in the 5′ untranslated region (UTR) of *NOTCH2NLC*.[1] While this article provides a concise overview of NIID, encompassing its clinical manifestations, age at onset, disease progression, diagnostic criteria, causative gene, and prognosis, our present study delves into a contentious issue: do the expanded GGC repeats within *NOTCH2NLC* directly cause a range of neurodegenerative diseases, including NIID? or do these conditions portray unique variations within the broader spectrum of NIID-related neurologic disorders?

Clinical Manifestations of Neuronal Intranuclear Inclusion Disease

In contrast to most neurodegenerative diseases that exhibit distinct and specific clinical manifestations, NIID displays remarkable symptom heterogeneity. Cognitive dysfunction is typically the primary and initial clinical manifestation in patients with NIID, which can encompass symptoms such as memory loss, personality changes, and abnormal mental behavior. Movement disorders can be the sole symptoms for the first decade, including parkinsonism, isolated

From the Department of Neurology (T.Z., L.B., H.C.), the Affiliated Hospital of Xuzhou Medical University; and Department of Neurology (L.B.), Xuzhou Medical University, China.

Go to Neurology.org/NG for full disclosures. Funding information is provided at the end of the article.

The Article Processing Charge was funded by Project supported by the Affiliated Hospital of Xuzhou Medical University.

Glossary

AD = Alzheimer disease; **ALS** = amyotrophic lateral sclerosis; **CMT** = Charcot-Marie-Tooth; **cSVD** = cerebral small vessel disease; **DWI** = diffusion-weighted imaging; **ET** = essential tremor; **FXTAS** = fragile X-associated tremor/ataxia syndrome; **NIID** = neuronal intranuclear inclusion disease; **NRED** = N-terminal domain repeat expansion disorder; **OPDM** = oculopharyngodistal myopathy; **PD** = Parkinson disease; **UTR** = untranslated region.

tremors, ataxia, and others; however, chorea has never been observed in NIID. Muscle weakness is also quite common, but the degree and pattern of muscle weakness can vary considerably from person to person. The majority of patients display characteristics of peripheral neuropathy, typically presenting with distal limb weakness and potential sensory impairments. Nevertheless, a small subset of patients primarily suffer proximal muscle weakness along with symptoms, such as ptosis, bulbar paralysis, and elevated muscle enzyme levels, indicating muscle involvement. Autonomic dysfunction is highly prominent in patients with NIID, although it is typically not recognized as the initial symptom, including orthostatic hypotension, urinary incontinence, and miosis. Moreover, paroxysmal symptoms are the most frequent and unique clinical manifestations of NIID, including alterations in consciousness, stroke-like attacks, encephalopathic episodes, and generalized convulsions.[2,3] Furthermore, we have previously documented that patients with NIID frequently present with various extraneurologic symptoms, including visual impairment, hearing loss, irritating dry cough, and persistent nausea.[4] Common neurologic and nonneurologic symptoms associated with NIID are elaborated in Figure 1.

Onset, Course, and Prognosis of Neuronal Intranuclear Inclusion Disease

The age at onset for this disease displays significant variation, spanning from infancy to later adulthood.[5-7] In Asia, the majority of NIID cases are observed in adults[8,9]; however, in the White population, it seems to be more common in infants and adolescents.[10-12] Individuals with NIID onset characterized by predominant dementia symptoms often experience a later manifestation, typically occurring within the age range of 43–55 years. Conversely, instances where muscle weakness is the prominent feature tend to show an earlier onset, usually falling between the ages of 16 and 39 years.[9]

In most cases, initial symptoms tend to progress slowly, and new symptoms may gradually appear over time, resulting in a combination of clinical manifestations. The accumulation of symptoms often results in an increased disability burden for patients.[9] However, in some cases, NIID may progress in paroxysms with acute or subacute attacks of encephalopathy. Episodic encephalopathy has been reported often in conjunction with stressful events, such as surgery, trauma, and infection.[13,14]

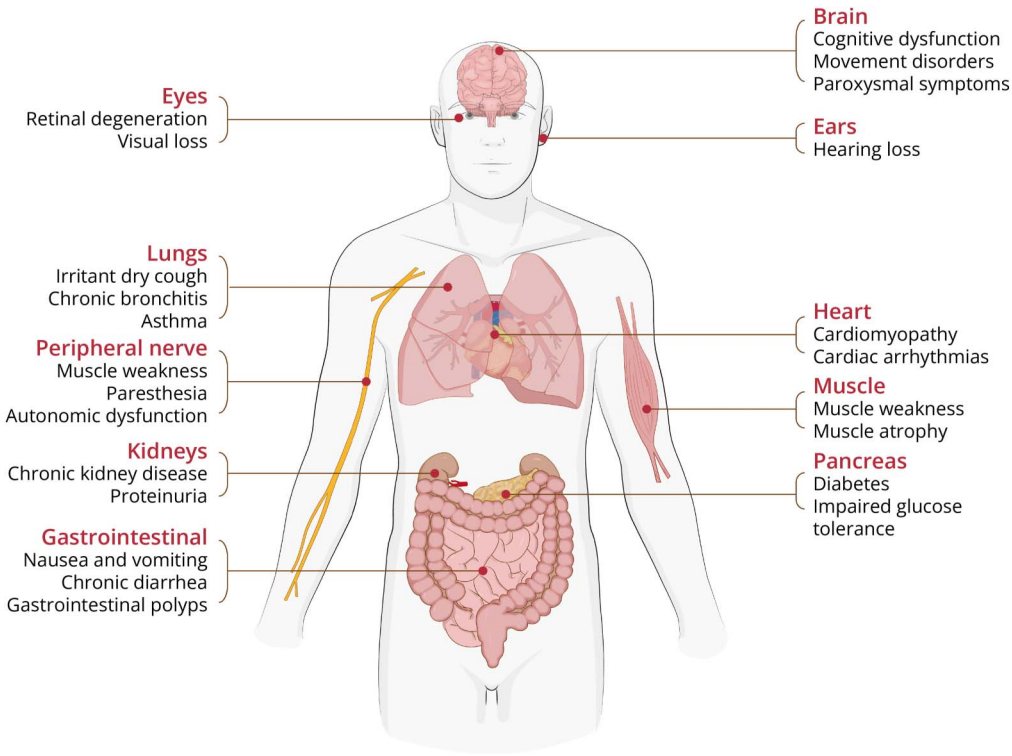
Currently, NIID lacks a specific targeted treatment, and there is a paucity of interventions to decelerate disease progression. However, most patients respond well to symptomatic treatment, at least during the initial phases of the condition. For instance, individuals with NIID-related parkinsonism phenotype often find relief through deep brain stimulation surgery or levodopa treatment, while those with NIID-related seizures can be effectively managed with carbamazepine.[15] Unfortunately, as the disease progresses, most patients will ultimately succumb to pneumonia.[12,16,17]

Causative Gene of Neuronal Intranuclear Inclusion Disease

In 2019, researchers in Japan and China identified the repetitive GGC expansion within the 5′ UTR of *NOTCH2NLC* as the genetic anomaly associated with NIID.[5-7] Nonetheless, a thorough examination of patients with NIID of European descent unveiled a rarity of repetitive GGC expansions in *NOTCH2NLC*, hinting that this particular expansion might not be the exclusive factor contributing to the onset of NIID or the development of neuronal intranuclear inclusions.[19]

NOTCH2NLC, along with *NOTCH2NLA* and *NOTCH2NLB*, constitutes 3 human-specific genes derived from *NOTCH2*, all situated on chromosome 1q21.1. These genes have been found to have high expression levels in the human brain and have been implicated in the evolution of the human brain.[19] Previously published data indicate that the lengths of GGC repeats within *NOTCH2NLC* in healthy controls range from 4 to 43 worldwide, with a narrower range of 4–41 in China.[5-7,20] Pathogenic GGC expansions are characterized by repeat sizes ranging from 60 to 517, and different sizes may lead to different clinical phenotypes. Individuals exhibiting the muscle weakness phenotype typically display larger GGC expansions, often exceeding 200 in size, with the largest expansion observed reaching 517. These patients also exhibit a higher frequency of GGA trinucleotide interruptions and fewer AGC trinucleotide interruptions. The parkinsonism phenotype features smaller repeat sizes, typically below 100, along with fewer GGA trinucleotide interruptions and an increased prevalence of AGC trinucleotide interruptions in these patients. Individuals with the dementia-dominant phenotype often display GGC repeat sizes and GGA trinucleotide interruption frequencies within a range typically spanning from 100 to 200 repeats.[3,21] Intermediate repeats, ranging from 42

Figure 1 Neurologic and Extraneurologic Clinical Symptoms in Patients With NIID



The clinical presentations of NIID vary significantly. Individuals affected by NIID may exhibit symptoms or signs that involve nearly all bodily systems, including the nervous system, respiratory system, circulation system, urinary system, and digestive system. NIID = neuronal intranuclear inclusion disease.

to 58, have been reported in patients with neurodegenerative dementias and leukoencephalopathy.[22,23] Specific details and frequencies of these intermediate repeats are currently unavailable, making it challenging to ascertain whether these patients should also receive a diagnosis of NIID, but one thing for certain is that intermediate repeats were not observed in healthy controls.[5-7,20]

Pathologic Mechanism of Neuronal Intranuclear Inclusion Disease

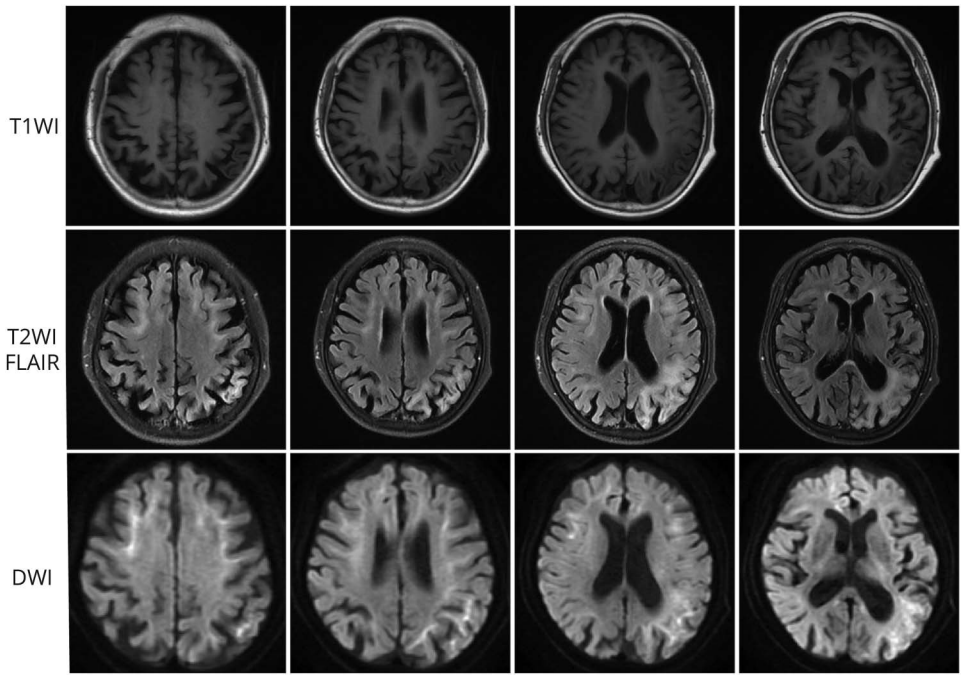
Two studies delved into the role of repeat expansions of GGC within the 5′UTR (5′ untranslated region) of *NOTCH2NLC* mRNA. These expansions can result in the translation of a polyglycine protein termed uN2CpolyG, which has been linked to neurotoxic effects observed in both in vivo and in vitro experiments.[24] In addition, uN2CpolyG toxicity was associated with retinal degeneration and impaired locomotion in transgenic Drosophila models expressing 100 GGC repeats.[25] Moreover, the elongated GGC repeat RNA can also undergo translation into 2 distinct repeat proteins: polyalanine (polyA) and polyarginine (polyR), alongside polyglycine. This translation process happens through a noncanonical mechanism referred to as repeat-associated non-ATG (RAN) translation.[26]

Diagnosis of Neuronal Intranuclear Inclusion Disease

In the initial case reports, the diagnosis of NIID largely relied on autopsy, rectal biopsy, and nerve biopsy methods.[27,28] However, Sone discovered that skin biopsies could provide a reliable pathologic diagnosis for NIID by identifying ubiquitin or p62-positive intranuclear inclusions, which resembled those in size, shape, and component from the brain (Figure 2).[29] Concurrently, Sone also reported high signal within both hemispheric cerebral white matter on T2 and fluid-attenuated inversion recovery sequences, as well as a distinctive high signal at the boundary between the cortex and medulla on diffusion-weighted imaging (DWI), as illustrated in Figure 3.[30] The specific change in the DWI of brain MRI has been consistently validated in numerous patients with NIID, making it a strong diagnostic indicator.

As research in the field of NIID continues to progress, there have been increasing concerns about the specificity and sensitivity of both imaging and pathologic diagnostic methods. Skin biopsy-detected pathologic inclusions can be seen in other GGC repeat expansion disorders, such as fragile X-associated tremor/ataxia syndrome (FXTAS) and oculopharyngodistal myopathy (OPDM).[31,32] Furthermore, the sensitivity of neuroimaging diagnosis varies with clinical

Figure 2 Characteristic MRI Findings in a Patient With NIID



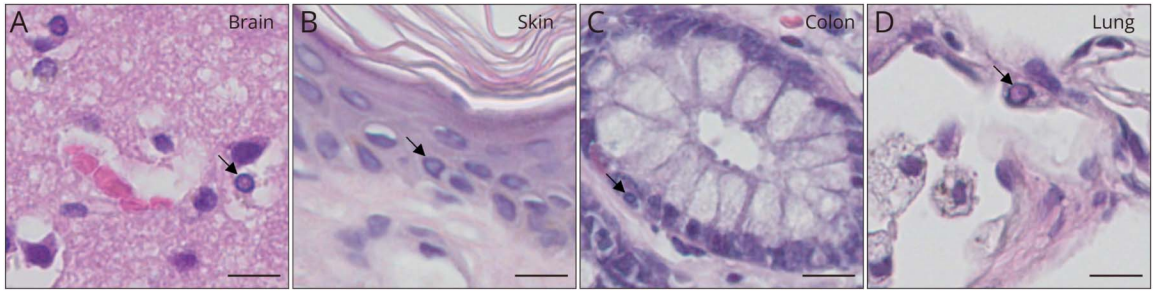
The brain MRI of a NIID patient with progressive cognitive decline exhibited distinct abnormalities: T1WI revealed hypointensity in the white matter of the left temporoparietal lobe. T2WI displayed hyperintensity in the white matter of both frontal lobes and the left parietal lobes. DWI showed bilateral frontal and left temporoparietal corticomedullary junction hyperintensity. NIID = neuronal intranuclear inclusion disease.

symptoms. Patients with dementia have a high DWI abnormality detection rate, up to 93.4%. However, those with parkinsonism or muscle weakness symptoms may not display typical imaging findings, with rates of 86.4% and 50%, respectively.[2] In addition, similar DWI abnormalities can also be observed in FXTAS.[33] Hence, neither pathologic nor imaging diagnosis can serve as a reliable basis for confirming the diagnosis of NIID, and for a certain duration, alongside histopathologic and imaging assessments, diagnosing NIID also necessitates the genetic exclusion of other trinucleotide repeat disorders. Until 2019, the primary diagnostic method for NIID in Asian populations shifted to genetic testing for the expansion of GGC repeats within *NOTCH2NLC*.[5-7]

A Controversial Issue Between Neuronal Intranuclear Inclusion Disease and Notch2 N-Terminal Domain Repeat Expansion Disorder

Recently, multiple studies have documented the existence of GGC repeat expansions in the 5′ UTR of *NOTCH2NLC* among patients diagnosed with Alzheimer disease (AD),[23] Parkinson disease (PD),[34,35] essential tremor (ET),[20] amyotrophic lateral sclerosis (ALS),[36] and OPDM.[37] Some of these authors have been quite assertive in suggesting the expansions of GGC repeats in *NOTCH2NLC* as a novel pathogenic gene

Figure 3 Detection of Eosinophilic Intranuclear Inclusions in Various Organs



(A) Brain tissue from a patient with NIID experiencing an encephalopathic episode displaying eosinophilic inclusions within microglia, visualized via hematoxylin and eosin (HE) staining. (B) Skin biopsy from a NIID patient with cognitive dysfunction revealing eosinophilic inclusions within squamous epithelial cell nuclei. (C) Tissue specimens from a previous colon surgery of a patient with NIID presenting with tremor, depicting eosinophilic inclusions within colonic mucosal epithelial cell nuclei. (D) Surgical biopsy of pulmonary nodules from a NIID patient with irritating cough, demonstrating eosinophilic inclusions within alveolar epithelial cells. NIID = neuronal intranuclear inclusion disease.

responsible for these diseases, proposing the concept of Notch2 N-terminal domain repeat expansion disorder (NRED).[38] Given the complex and varied clinical presentations of NIID, the emergence of the concept of NRED has brought forth a contentious question: do the expanded GGC repeats within *NOTCH2NLC* directly cause a range of neurodegenerative diseases, including NIID? or do these conditions portray unique variations within the broader spectrum of NIID-related neurologic disorders?

Distinguishing between NIID and NRED is of utmost importance, as an accurate diagnosis plays a critical role in shaping treatment strategies and predicting the disease's prognosis. Given the high likelihood of multiple neurologic dysfunctions advancing in most patients with NIID, the severity of their condition can be more pronounced, resulting in heightened suffering and greater challenges compared with individuals with other neurologic disorders. We will systematically review the research on *NOTCH2NLC* 5′ UTR GGC repeat expansions and their association with related neurologic diseases, explaining the limitations of the relevant reports. Furthermore, we will integrate subsequent studies to further demonstrate that these individuals actually experienced distinct clinical phenotypes associated with NIID.

NIID-Related Dementia Phenotype or Alzheimer Disease/Frontotemporal Dementia Caused by GGC Repeat Expansions in *NOTCH2NLC*

In a research analysis involving 1,004 patients diagnosed with neurodegenerative dementias, 4 individuals initially diagnosed with either AD or FTD were found to carry pathogenic GGC expansions within *NOTCH2NLC*. However, characteristic DWI anomalies associated with NIID were not observed in these patients. Instead, significant leukoencephalopathy was detected in 3 of them using T2 MRI. The challenge lies in determining the appropriate diagnosis for these cases, especially because dementia is a prominent symptom in NIID and often the primary reason for seeking medical evaluation. Nevertheless, 2 patients died before further evaluation, and the 2 others refrained from further assessments, making it insufficient to diagnose them with AD or FTD without pathologic evidence to exclude NIID.[23]

In another study, researchers reported GGC expansions within *NOTCH2NLC* in 2 of 140 families affected by AD.[7] When re-evaluating 11 individuals displaying symptoms from these two-family lineages, researchers observed not only prominent dementia symptoms but also other atypical features not frequently observed in AD, such as tremor, parkinsonism, autonomic dysfunction, altered consciousness, and encephalitic episodes. In addition, 2 members from these families displayed severe leukoencephalopathy with DWI hyperintensity. Skin biopsies taken from symptomatic family members showcased the existence of eosinophilic inclusions positive for p62 and ubiquitin. Based on these findings, the patients from these families were ultimately diagnosed with the NIID-related dementia phenotype rather than the initial diagnosis of Alzheimer disease.[7] Hence, when presented with a patient with dementia harboring GGC repeat expansion in *NOTCH2NLC*, diagnosing the NIID-related dementia phenotype might be more fitting than AD. A subsequent skin biopsy can provide additional confirmation for the diagnosis.

NIID-Related Tremor Phenotype or Essential Tremor Caused by GGC Repeat Expansions in *NOTCH2NLC*

In a retrospective study,[20] it was found that GGC repeat expansions within *NOTCH2NLC* were present in 5.6% (11/197 pedigrees) of familial cases of ET. The expanded GGC repeat sizes varied from 60 to 250 repeats. During a 10-year follow-up, these patients did not exhibit typical clinical symptoms or positive imaging findings of NIID. Consequently, the researcher suggested that these patients ought to receive a clinical diagnosis of ET, despite the presence of ubiquitin-positive and p62-positive intranuclear inclusions found in skin tissues of 3 genetically positive probands in the study.[20]

However, according to 2 more recent studies, 2 "ET" patients with elongated GGC repeats in *NOTCH2NLC* exhibited continuous cognitive decline over a 4- and 10-year follow-up period. In addition, they displayed characteristic histopathologic and radiologic traits associated with NIID.[39,40] These compelling findings have led us to lean toward diagnosing these patients with NIID. More compelling evidence was provided by another study that "ET" is an independent clinical manifestation of NIID.[41] Among 602 individuals initially diagnosed with ET, 10 patients harboring NOTCH2NLC-GGC repeat expansions were identified. Systematic re-evaluation of seven of these patients revealed cognitive decline, systemic absence of reflexes, and decreased nerve conduction, which are not typically seen in patients with ET. Furthermore, among them, 3 demonstrated hyperintense in the corticomedullary junction on DWI, while intranuclear inclusions were detected in all 4 probands who underwent skin biopsies. As a result, after re-evaluation, the clinical diagnosis of all these probands was altered from ET to NIID.[41]

Considering these findings, a thorough systematic re-evaluation and extended follow-up for individuals diagnosed with "ET" but presenting GGC repeat expansions in *NOTCH2NLC* are strongly recommended. In such cases, considering the tremor phenotype associated with NIID might be more appropriate for diagnosis.

NIID-Related Parkinsonism Phenotype or Parkinson Disease Caused by GGC Repeat Expansions in *NOTCH2NLC*

Two separate research studies investigated 1,000 and 1,011 patients with PD, respectively, and found that 13 and 11 of them carried expansions of GGC repeats in *NOTCH2NLC*. However, none of these individuals displayed additional clinical symptoms or characteristic radiologic features of NIID even after several years of follow-up. As a result, they concluded that it would be more appropriate to diagnose all these patients with PD attributed to GGC repeat expansions in *NOTCH2NLC* instead of the parkinsonism phenotype related to NIID.[34,35] However, these studies have certain limitations. First, they did not consider that patients with NIID-related parkinsonism might not show typical corticomedullary high signal on DWI, as previously reported in case studies and series.[2,7,42,43] Thus, the lack of typical MRI features should not be considered grounds for excluding the possibility of NIID. Second, only a small subset of patients from both studies underwent either skin or postmortem brain biopsies. Therefore, based on the evidence provided in these studies, it is inadequate to definitively assert that *NOTCH2NLC* GGC repeat expansions are exclusively linked to PD.

A recent study examined 941 clinically established patients with PD and found 3 of them carrying the pathogenic GGC expansion. These patients did not exhibit additional symptoms or display typical imaging characteristics associated with NIID. However, intriguingly, skin biopsies from all 3 patients showed intranuclear inclusions; however, pathologic hallmarks of PD (p-α-nucleotide depositions) in dermal nerve fibers were absent. This indicates that patients with PD initially diagnosed with *NOTCH2NLC* GGC expansions display the parkinsonism phenotype associated with NIID.[44] Therefore, the distinct histopathologic features between NIID and PD serve as pivotal factors in distinguishing between these conditions.

NIID-Related Amyotrophic Lateral Sclerosis-Like Phenotype or Amyotrophic Lateral Sclerosis Caused by GGC Repeat Expansions in *NOTCH2NLC*

In a recent retrospective study assessing the involvement of GGC repeats in *NOTCH2NLC* in ALS, researchers found that 4 of 545 patients with ALS carried pathogenic GGC repeat expansions in *NOTCH2NLC*, with the size of these repeats varying between 44 and 143 in affected individuals. Among these patients, 2 died, precluding further evaluation. However, the remaining 2 patients underwent systematic re-evaluation, which revealed some atypical features not commonly seen in ALS. These 2 patients exhibited symptoms, such as tremor, numbness, and autonomic dysfunction, which are rarely uncommonly in conventional ALS cases. As the disease advanced, they displayed decreased sensory and motor nerve conduction velocities, indicating concomitant peripheral nerve damage. In addition, one of the patients presented cognitive dysfunction, along with diffuse leukoencephalopathy and bilateral DWI high signal in the corticomedullary junction. Skin biopsies from both patients exhibited eosinophilic inclusions positive for p62 and ubiquitin. Based on these findings, they did not arbitrarily treat *NOTCH2NLC* mutation as a novel ALS-causative gene; instead, the study's findings imply that *NOTCH2NLC* GGC repeat expansions might contribute to a particular ALS phenotype, demonstrating similarities with NIID characteristics.[36] This underscores the importance of considering NIID-related features when assessing ALS patients with pathogenic *NOTCH2NLC* GGC repeat expansions.

The possibility of coexisting NIID with ALS requires further discussion. In a reported case, a patient previously diagnosed with ALS exhibited intranuclear inclusions with p62 positivity in skin biopsy samples from adipocytes and sweat gland cell.[45] In the absence of genetic results, the presence of these inclusions in the skin tissues led researchers to erroneously assume that the patient could be diagnosed with NIID. Postmortem autopsy unveiled both p62-positive nuclear inclusions and pTDP-43–positive cytoplasmic inclusions, recognized as pathologic ALS hallmarks. However, the absence of a distinctive concentration of p62-positive nuclear inclusions in regions densely populated with large motor neurons, coupled with the lack of coexistence of nuclear and cytoplasmic inclusions within these neurons, prompted researchers to contemplate the co-occurrence of both NIID and ALS in this patient.[45]

While the autopsy findings in this patient were comprehensively described, it is essential to note that intranuclear inclusions showing p62 positivity should not be considered a specific pathologic hallmark exclusive to NIID. It is essential to note that intranuclear inclusions exhibiting p62 positivity should not be solely indicative or unique to NIID as a specific diagnostic feature. They can also be present in various nucleotide repeat expansion disorders, including patients with *C9orf72*-mediated ALS.[46-48] The autopsy findings from 14 individuals with C9orf72-mediated ALS revealed the presence of p62-positive nuclear inclusions and pTDP-43-positive cytoplasmic inclusions in motor neurons. These inclusions did not colocalize, aligning with the observations outlined in the study.[45] Therefore, the absence of identification of GGC repeat expansion of *NOTCH2NLC* in Sugiyama's case raises questions about the reliability of diagnosing NIID, necessitating an assessment of GGGGCC repeat expansions in *C9orf72* in this patient.

The GGC repeat expansion within *NOTCH2NLC* is notably associated with neurologic manifestations resembling ALS symptoms observed in NIID, rather than being primarily

attributed as a direct cause of ALS. Careful consideration is needed when diagnosing a concurrent case of NIID and ALS, and the exclusion of expansion of GGGGCC repeats in the *C9orf72* is necessary.

NIID-Related Oculopharyngodistal Myopathy Phenotype or Oculopharyngodistal Myopathy Caused by GGC Repeat Expansions in *NOTCH2NLC*

Recently, 2 studies have reported an association between GGC repeat expansion in *NOTCH2NLC* and OPDM. In 1 study, it was identified that among a cohort of 24 patients with OPDM, GGC repeat expansions in *NOTCH2NLC* were present in 5 cases. One patient exhibited diffuse leukoencephalopathy, along with corticomedullary high signal on DWI. Among the 4 patients who underwent nerve conduction examinations, all individuals exhibited diminished motor nerve conduction velocities and reduced compound muscle action potential amplitudes. p62-positive intranuclear inclusions were detected in 1 patient through skin biopsy. In this study, *NOTCH2NLC* was suggested as a causative factor in OPDM, with these patients classified as OPDM type 3, disregarding the presence of radiologic abnormalities and subclinical peripheral neuropathy unrelated to myopathy in these individuals.[49]

In another separate investigation, it was identified that 7 individuals initially diagnosed with OPDM harbored GGC repeat expansions in *NOTCH2NLC*, all of whom had p62-positive intranuclear inclusions within myocytes. Among them, 3 patients presented with ataxia, and 1 showed tremor, in addition, to the characteristic clinical manifestations of OPDM. Among these cases, 3 individuals exhibited ataxia, while 1 displayed rest tremors, alongside the typical clinical features associated with OPDM. Two of 5 patients exhibited leukoencephalopathy with DWI abnormalities. The neurologic symptoms, both peripheral and central, along with imaging irregularities observed in these patients, bear a resemblance to those typically observed in NIID cases.[32] Therefore, it is more probable that these patients exhibit a phenotype resembling OPDM associated with NIID rather than being categorized specifically as *NOTCH2NLC*-associated OPDM.

The above findings highlight the importance of considering the possible overlap between NIID and OPDM when evaluating patients with GGC repeat expansions in *NOTCH2NLC*. The clinical and radiologic features may be indicative of a broader spectrum of neurodegenerative disorders, and a comprehensive assessment is necessary to accurately diagnose and manage these patients.

NIID-Related Charcot-Marie-Tooth-Like Phenotype or Charcot-Marie-Tooth Caused by GGC Repeat Expansions in *NOTCH2NLC*

In a study encompassing 127 patients with hereditary neuropathy of unknown genetic origin, researchers identified GGC repeat expansions in *NOTCH2NLC* among 7 patients initially diagnosed with Charcot-Marie-Tooth (CMT). All 7 individuals showed axonal neuropathy with predominantly sensory impairment. MRI of the brain was conducted on 5 patients, yet no distinctive NIID-related findings were observed. However, 2 biopsy specimens taken from the skin revealed the existence of eosinophilic intranuclear inclusions positive for p62 and ubiquitin. In addition, 2 of 7 patients had a familial background related to NIID.[50] Considering these results, diagnosing these patients with a CMT-like phenotype associated with NIID appears appropriate.

Furthermore, previous reports have suggested that peripheral neuropathy related to NIID frequently manifests as a demyelinating neuropathy primarily affecting motor function. Therefore, the phenotypic spectrum of NIID-related peripheral neuropathy may encompass both predominant demyelinating motor neuropathy and predominant axonal sensory neuropathy, necessitating further studies for confirmation.[51] These findings highlight the significance of considering NIID-related features when evaluating patients with unclear genetic causes of inherited neuropathies. The clinical presentation of NIID-related peripheral neuropathy may vary, and it is crucial to recognize its diverse phenotypes to ensure accurate diagnosis and appropriate management for affected individuals.

NIID-Related Vascular Leukoencephalopathy Phenotype or Vascular Leukoencephalopathy Caused by GGC Repeat Expansions in *NOTCH2NLC*

A study investigated the potential link between *NOTCH2NLC* GGC repeat expansions and cerebral small vessel disease (cSVD) in genetically unresolved vascular leukoencephalopathy cases. Genetic data from 814 patients with cSVD were analyzed, revealing pathogenic GGC repeat expansions in *NOTCH2NLC* in 9 patients (1.11% of cases), ranging from 41 to 98 repeats.[52] Among these 9 patients, 6 primarily sought medical attention for nonspecific symptoms such as headaches and dizziness, which may not necessarily be related to stroke. Conversely, 3 of them exhibited stroke-related symptoms such as slurred speech, hemiparesis, and hemiparesthesia. Unfortunately, all these patients had risk factors for cerebrovascular diseases, such as diabetes and hypertension, making it challenging to definitively attribute their cerebrovascular

disease to GGC repeat expansions or other high-risk factors. Furthermore, apart from the extensive leukoencephalopathy, these 9 patients did not display the typical radiologic characteristics seen in other cerebral small vessel diseases, such as multiple lacunar infarctions, cerebral microbleeds, and enlargement of perivascular spaces.[52] Hence, based on this study, it is challenging to establish a causal relationship between the expansion of GGC repeats in *NOTCH2NLC* and the development of cSVD.

Almost at the same time, another study screened 197 patients with unexplained cSVD and identified 6 patients carrying the *NOTCH2NLC* GGC repeat expansion. These patients displayed gait issues, cognitive decline, and distinct cSVD traits. Notably, 4 individuals displayed typical NIID-related hyperintensity in the corticomedullary junction, while 1 individual's biopsy revealed eosinophilic inclusions positive for ubiquitin and p62 within the cells of the capillary and sweat gland, suggesting a role of *NOTCH2NLC* GGC repeat expansions in causing cerebral small vessel disease. Finally, they concluded that patients exhibiting cSVD should be considered for the possibility of NIID, particularly those demonstrating characteristic neuroimaging features associated with NIID.[53]

In clinical practice, patients with NIID can indeed exhibit stroke-like episodes, and newly occurring subcortical infarctions can be detected on MRI.[30,54] In addition, typical cerebral small vessel disease imaging features such as extensive white matter disease, brain atrophy, and subcortical infarctions are also commonly observed in individuals with NIID.[55] Furthermore, it has been documented that the majority of individuals affected by NIID exhibit localized reductions in cerebral blood perfusion as observed through single-photon emission CT imaging.[9] A more compelling validation of the potential role of GGC repeat expansions within *NOTCH2NLC* in contributing to a phenotype of cSVD is provided by an autopsy study of a NIID patient with a stroke-like episode. This study unveiled pathologic observations that could indicate compromised integrity of the blood-brain barrier and disturbances in the regulation of cerebral blood flow.[56] Thus, patients with NIID are very likely to present with clinical phenotypes of cerebral small vessel disease.

Conclusions

As previously discussed in the literature, the attribution of GGC repeat expansions in *NOTCH2NLC* as the primary factor behind various neurodegenerative diseases appears to have harbored some inaccuracies. First, certain earlier studies erroneously relied on the absence of MRI abnormalities as an absolute criterion for excluding the possibility of NIID, which led to potential misclassifications. Second, these studies might have inadvertently overlooked additional clinical manifestations that are not typically associated with other related neurodegenerative conditions. In stark contrast, a multitude of subsequent investigations have amassed compelling pathologic evidence affirming that these individuals indeed present distinct clinical phenotypes linked to NIID.

Although screening GGC expansions of *NOTCH2NLC* in preexisting DNA sample repositories has the potential to advance our knowledge of the disease's prevalence and breadth and to uncover undiagnosed cases, it is important to note that we should not automatically assume it to be a causative gene for related diseases, which may lead to profound alterations in disease incidence, mutation frequency, and clinical presentations. We also recommend that patients with neurodegenerative conditions exhibiting hallmark features of NIID, such as paroxysmal symptoms and indications of multisystem involvement, should undergo genetic testing for GGC repeats in *NOTCH2NLC*, regardless of whether they show DWI high-signal abnormalities in the corticomedullary junction or intranuclear inclusions in their biopsy specimens.

Study Funding

Open research project of Jiangsu Provincial Key Laboratory (XZSYSKF2021015).

Disclosure

The authors report no relevant disclosures. Go to Neurology.org/NG for full disclosures.

Publication History

Received by *Neurology: Genetics* September 14, 2023. Accepted in final form January 5, 2024. Submitted and externally peer reviewed. The handling editor was Editor Stefan M. Pulst, MD, Dr med, FAAN.

Appendix Authors

Name	Location	Contribution
Tao Zhang, MD	Department of Neurology, the Affiliated Hospital of Xuzhou Medical University, China	Drafting/revision of the manuscript for content, including medical writing for content
Lei Bao, PhD	Department of Neurology, the Affiliated Hospital of Xuzhou Medical University; Department of Neurology, Xuzhou Medical University, China	Drafting/revision of the manuscript for content, including medical writing for content; analysis or interpretation of data
Hao Chen, MD	Department of Neurology, the Affiliated Hospital of Xuzhou Medical University, China	Drafting/revision of the manuscript for content, including medical writing for content

References

1. Tai H, Wang A, Zhang Y, et al. Clinical features and classification of neuronal intranuclear inclusion disease. *Neurol Genet.* 2023;9(2):e200057. doi:10.1212/NXG.0000000000200057
2. Tian Y, Zhou L, Gao J, et al. Clinical features of NOTCH2NLC-related neuronal intranuclear inclusion disease. *J Neurol Neurosurg Psychiatry*. 2022;93(12):1289-1298. doi:10.1136/jnnp-2022-329772
3. Huang XR, Tang BS, Jin P, Guo JF. The phenotypes and mechanisms of NOTCH2NLC-Related GGC repeat expansion disorders: a comprehensive review. *Mol Neurobiol.* 2022;59(1):523-534. doi:10.1007/s12035-021-02616-2

4. Chen H, Lu L, Wang B, et al. Re-defining the clinicopathological spectrum of neuronal intranuclear inclusion disease. *Ann Clin Transl Neurol.* 2020;7(10):1930-1941. doi: 10.1002/acn3.51189
5. Ishiura H, Shibata S, Yoshimura J, et al. Noncoding CGG repeat expansions in neuronal intranuclear inclusion disease, oculopharyngodistal myopathy and an overlapping disease. *Nat Genet.* 2019;51(8):1222-1232. doi:10.1038/s41588-019-0458-z
6. Sone J, Mitsuhashi S, Fujita A, et al. Long-read sequencing identifies GGC repeat expansions in NOTCH2NLC associated with neuronal intranuclear inclusion disease. *Nat Genet.* 2019;51(8):1215-1221. doi:10.1038/s41588-019-0459-y
7. Tian Y, Wang JL, Huang W, et al. Expansion of human-specific GGC repeat in neuronal intranuclear inclusion disease-related disorders. *Am J Hum Genet.* 2019; 105(1):166-176. doi:10.1016/j.ajhg.2019.05.013
8. Liang H, Wang B, Li Q, et al. Clinical and pathological features in adult-onset NIID patients with cortical enhancement. *J Neurol.* 2020;267(11):3187-3198. doi:10.1007/s00415-020-09945-7
9. Sone J, Mori K, Inagaki T, et al. Clinicopathological features of adult-onset neuronal intranuclear inclusion disease. *Brain*. 2016;139(Pt 12):3170-3186. doi:10.1093/brain/aww249
10. O'Sullivan JD, Hanagasi HA, Daniel SE, Tidswell P, Davies SW, Lees AJ. Neuronal intranuclear inclusion disease and juvenile parkinsonism. *Mov Disord.* 2000;15(5): 990-995. doi:10.1002/1531-8257(200009)15:5<990::aid-mds1035>3.0.co;2-i
11. Wiltshire KM, Dunham C, Reid S, Auer RN, Suchowersky O. Neuronal intranuclear inclusion disease presenting as juvenile Parkinsonism. *Can J Neurol Sci.* 2010;37(2): 213-218. doi:10.1017/s031716710000994x
12. Pilson K, Farrell M, Lynch B, Devaney D. A case of juvenile onset neuronal intranuclear inclusion disease with a negative antemortem skin biopsy. *Pediatr Dev Pathol.* 2018;21(5):494-496. doi:10.1177/1093526617724293
13. Chen L, Wu L, Li S, et al. A long time radiological follow-up of neuronal intranuclear inclusion disease: two case reports. *Medicine (Baltimore)*. 2018;97(49):e13544. doi: 10.1097/MD.0000000000013544
14. Xiao F, Tian X, Wang XF. Cerebral atrophy and leukoencephalopathy in a young man presenting with encephalitic episodes. *JAMA Neurol.* 2018;75(12):1563-1564. doi: 10.1001/jamaneurol.2018.2333
15. Fujita K, Osaki Y, Miyamoto R, et al. Neurologic attack and dynamic perfusion abnormality in neuronal intranuclear inclusion disease. *Neurol Clin Pract.* 2017;7(6): e39-e42. doi:10.1212/CPJ.0000000000000389
16. Kimber TE, Blumbergs PC, Rice JP, et al. Familial neuronal intranuclear inclusion disease with ubiquitin positive inclusions. *J Neurol Sci.* 1998;160(1):33-40. doi: 10.1016/s0022-510x(98)00169-5
17. Malandrini A, Villanova M, Tripodi S, et al. Neuronal intranuclear inclusion disease: neuropathologic study of a case. *Brain Dev.* 1998;20(5):290-294. doi:10.1016/s0387-7604(98)00032-1
18. Chen Z, Yan Yau W, Jaunmuktane Z, et al. Neuronal intranuclear inclusion disease is genetically heterogeneous. *Ann Clin Transl Neurol.* 2020;7(9):1716-1725. doi: 10.1002/acn3.51151
19. Suzuki IK, Gacquer D, Van Heurck R, et al. Human-specific NOTCH2NL genes expand cortical neurogenesis through delta/notch regulation. *Cell*. 2018;173(6): 1370-1384.e16. doi:10.1016/j.cell.2018.03.067
20. Sun QY, Xu Q, Tian Y, et al. Expansion of GGC repeat in the human-specific NOTCH2NLC gene is associated with essential tremor. *Brain*. 2020;143(1):222-233. doi:10.1093/brain/awz372
21. Chen Z, Xu Z, Cheng Q, et al. Phenotypic bases of NOTCH2NLC GGC expansion positive neuronal intranuclear inclusion disease in a Southeast Asian cohort. *Clin Genet.* 2020;98(3):274-281. doi:10.1111/cge.13802
22. Okubo M, Doi H, Fukai R, et al. GGC repeat expansion of NOTCH2NLC in adult patients with leukoencephalopathy. *Ann Neurol.* 2019;86(6):962-968. doi:10.1002/ana.25586
23. Jiao B, Zhou L, Zhou Y, et al. Identification of expanded repeats in NOTCH2NLC in neurodegenerative dementias. *Neurobiol Aging*. 2020;89:142 e1–e7. doi:10.1016/j.neurobiolaging.2020.01.010
24. Boivin M, Deng J, Pfister V, et al. Translation of GGC repeat expansions into a toxic polyglycine protein in NIID defines a novel class of human genetic disorders: the polyG diseases. *Neuron*. 2021;109(11):1825-1835.e5. doi:10.1016/j.neuron.2021.03.038
25. Yu J, Liufu T, Zheng Y, et al. CGG repeat expansion in NOTCH2NLC causes mitochondrial dysfunction and progressive neurodegeneration in Drosophila model. *Proc Natl Acad Sci U S A*. 2022;119(41):e2208649119. doi:10.1073/pnas.2208649119
26. Liu Q, Zhang K, Kang Y, et al. Expression of expanded GGC repeats within NOTCH2NLC causes behavioral deficits and neurodegeneration in a mouse model of neuronal intranuclear inclusion disease. *Sci Adv*. 2022;8(47):eadd6391. doi: 10.1126/sciadv.add6391
27. Goutieres F, Mikol J, Aicardi J. Neuronal intranuclear inclusion disease in a child: diagnosis by rectal biopsy. *Ann Neurol.* 1990;27(1):103-106. doi:10.1002/ana.410270117
28. Funata N, Maeda Y, Koike M, et al. Neuronal intranuclear hyaline inclusion disease: report of a case and review of the literature. *Clin Neuropathol.* 1990;9(2):89-96.
29. Sone J, Tanaka F, Koike H, et al. Skin biopsy is useful for the antemortem diagnosis of neuronal intranuclear inclusion disease. *Neurology*. 2011;76(16):1372-1376. doi: 10.1212/WNL.0b013e3182166e13
30. Sone J, Kitagawa N, Sugawara E, et al. Neuronal intranuclear inclusion disease cases with leukoencephalopathy diagnosed via skin biopsy. *J Neurol Neurosurg Psychiatry*. 2014;85(3):354-356. doi:10.1136/jnnp-2013-306084
31. Toko M, Ohshita T, Kurashige T, et al. FXTAS is difficult to differentiate from neuronal intranuclear inclusion disease through skin biopsy: a case report. *BMC Neurol.* 2021;21(1):396. doi:10.1186/s12883-021-02425-z
32. Ogasawara M, Eura N, Nagaoka U, et al. Intranuclear inclusions in skin biopsies are not limited to neuronal intranuclear inclusion disease but can also be seen in oculopharyngodistal myopathy. *Neuropathol Appl Neurobiol.* 2022;48(3):e12787. doi: 10.1111/nan.12787
33. Cabal-Herrera AM, Tassanakijpanich N, Salcedo-Arellano MJ, Hagerman RJ. Fragile X-associated tremor/ataxia syndrome (FXTAS): pathophysiology and clinical implications. *Int J Mol Sci.* 2020;21(12):4391. doi:10.3390/ijms21124391
34. Ma D, Tan YJ, Ng ASL, et al. Association of NOTCH2NLC repeat expansions with Parkinson disease. *JAMA Neurol.* 2020;77(12):1559-1563. doi:10.1001/jamaneurol.2020.3023
35. Shi CH, Fan Y, Yang J, et al. NOTCH2NLC intermediate-length repeat expansions are associated with Parkinson disease. *Ann Neurol.* 2021;89(1):182-187. doi:10.1002/ana.25925
36. Yuan Y, Liu Z, Hou X, et al. Identification of GGC repeat expansion in the NOTCH2NLC gene in amyotrophic lateral sclerosis. *Neurology*. 2020;95(24): e3394-e3405. doi:10.1212/WNL.0000000000010945
37. Ogasawara M, Iida A, Kumutpongpanich T, et al. CGG expansion in NOTCH2NLC is associated with oculopharyngodistal myopathy with neurological manifestations. *Acta Neuropathol Commun.* 2020;8(1):204. doi:10.1186/s40478-020-01084-4
38. Westenberger A, Klein C. Essential phenotypes of NOTCH2NLC-related repeat expansion disorder. *Brain*. 2020;143(1):5-8. doi:10.1093/brain/awz404
39. Ng ASL, Lim WK, Xu Z, et al. NOTCH2NLC GGC repeat expansions are associated with sporadic essential tremor: variable disease expressivity on long-term follow-up. *Ann Neurol.* 2020;88(3):614-618. doi:10.1002/ana.25803
40. Chen H, Lu L, Wang B, et al. Essential tremor as the early symptom of NOTCH2NLC gene-related repeat expansion disorder. *Brain.* 2020;143(7):e56. doi:10.1093/brain/awaa142
41. Yang D, Cen Z, Wang L, et al. Neuronal intranuclear inclusion disease tremor-dominant subtype: a mimicker of essential tremor. *Eur J Neurol.* 2022;29(2):450-458. doi:10.1111/ene.15169
42. Paviour DC, Revesz T, Holton JL, Evans A, Olsson JE, Lees AJ. Neuronal intranuclear inclusion disease: report on a case originally diagnosed as dopa-responsive dystonia with Lewy bodies. *Mov Disord.* 2005;20(10):1345-1349. doi:10.1002/mds.20559
43. Vermilion J, Johnson M, Srinivasan J, Mink JW. Neuronal intranuclear inclusion disease: longitudinal case report of motor and nonmotor symptoms. *J Child Neurol.* 2019;34(13):801-805. doi:10.1177/0883073819860566
44. Liu P, Yang D, Zhang F, et al. The role of NOTCH2NLC in Parkinson's disease: a clinical, neuroimaging, and pathological study. *Eur J Neurol.* 2022;29(6):1610-1618. doi:10.1111/ene.15283
45. Sugiyama A, Takeda T, Koide M, et al. Coexistence of neuronal intranuclear inclusion disease and amyotrophic lateral sclerosis: an autopsy case. *BMC Neurol.* 2021;21(1): 273. doi:10.1186/s12883-021-02306-5
46. Mann DM, Rollinson S, Robinson A, et al. Dipeptide repeat proteins are present in the p62 positive inclusions in patients with frontotemporal lobar degeneration and motor neurone disease associated with expansions in C9ORF72. *Acta Neuropathol Commun.* 2013;1:68. doi:10.1186/2051-5960-1-68
47. Lee SM, Asress S, Hales CM, et al. TDP-43 cytoplasmic inclusion formation is disrupted in C9orf72-associated amyotrophic lateral sclerosis/frontotemporal lobar degeneration. *Brain Commun.* 2019;1(1):fcz014. doi:10.1093/braincomms/fcz014
48. Davidson JM, Chung RS, Lee A. The converging roles of sequestosome-1/p62 in the molecular pathways of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). *Neurobiol Dis.* 2022;166:105653. doi:10.1016/j.nbd.2022.105653
49. Yu J, Deng J, Guo X, et al. The GGC repeat expansion in NOTCH2NLC is associated with oculopharyngodistal myopathy type 3. *Brain*. 2021;144(6):1819-1832. doi: 10.1093/brain/awab077
50. Liao YC, Chang FP, Huang HW, et al. GGC repeat expansion of NOTCH2NLC in Taiwanese patients with inherited neuropathies. *Neurology*. 2022;98(2):e199-e206. doi:10.1212/WNL.0000000000013008
51. Sone J, Hishikawa N, Koike H, et al. Neuronal intranuclear hyaline inclusion disease showing motor-sensory and autonomic neuropathy. *Neurology*. 2005;65(10): 1538-1543. doi:10.1212/01.wnl.0000184490.22527.90
52. Wang YC, Fan Y, Yu WK, et al. NOTCH2NLC expanded GGC repeats in patients with cerebral small vessel disease. *Stroke Vasc Neurol.* 2023;8(2):161-168. doi: 10.1136/svn-2022-001631
53. Liao YC, Wei CY, Chang FP, et al. NOTCH2NLC GGC repeat expansion in patients with vascular leukoencephalopathy. *Stroke*. 2023;54(5):1236-1245. doi:10.1161/STROKEAHA.122.041848
54. Lin P, Jin H, Yi KC, et al. A case report of sporadic adult neuronal intranuclear inclusion disease (NIID) with stroke-like onset. *Front Neurol.* 2020;11:530. doi: 10.3389/fneur.2020.00530
55. Liu YH, Chou YT, Chang FP, et al. Neuronal intranuclear inclusion disease in patients with adult-onset non-vascular leukoencephalopathy. *Brain*. 2022;145(9):3010-3021. doi:10.1093/brain/awac135
56. Yoshii D, Ayaki T, Wada T, et al. An autopsy case of adult-onset neuronal intranuclear inclusion disease with perivascular preservation in cerebral white matter. *Neuropathology*. 2022;42(1):66-73. doi:10.1111/neup.12778