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Clinicopathologic Characterization of 2 Individuals With *TBK1* Variants—1 Novel Splice Variant, 2 Proteinopathies

A Case Series

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Abstract

Objectives

Here, we report detailed clinicopathologic evaluation of 2 individuals with pathogenic variants in *TBK1*, including one novel likely pathogenic splice variant. We describe the striking diversity of clinical phenotypes among family members and also the brain and spinal cord neuropathology associated with these 2 distinct *TBK1* variants.

Methods

Two individuals with pathogenic variants in *TBK1* and their families were clinically characterized, and the probands subsequently underwent extensive postmortem neuropathologic examination of their brains and spinal cords.

Results

Multiple affected individuals within a single family were found to carry a previously unreported c.358+3A>G variant, predicted to alter splicing. Detailed histopathologic evaluation of our 2 *TBK1* variant carriers demonstrated distinct TDP-43 pathologic subtypes, but shared argyrophilic grain disease (AGD) tau pathology.

Discussion

Although all pathogenic *TBK1* variants are associated with TDP-43 pathology, the clinical and histologic features can be highly variable. Within one family, we describe distinct neurologic presentations which we propose are all caused by a novel c.358+3A>G variant. AGD is typically associated with older age, but it has been described as a copathologic finding in other *TBK1* variant carriers and may be a common feature in FTLD-TDP due to *TBK1*.

Introduction

Frontotemporal dementia (FTD) with motor neuron disease (MND) is a well-recognized phenotype in the frontotemporal lobar degeneration (FTLD) spectrum. Increasing use of genetic testing has led to identification of multiple pathogenic gene variants associated with this syndrome, making it important to fully characterize not only the range of potential clinical features but also the underlying neuropathologic findings. Here, we present 2 families, one with a novel *TBK1* variant predicted to alter splicing. We performed extensive neuropathologic characterization of the brain and spinal cord, highlighting the variability of TDP-43 neuropathology and possible coassociation with tau neuropathology.

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Standard Protocol Approvals, Registrations, and Patient Consents

The Institutional Review Board of the University of Washington (UW) approved this work. Consent-to-Disclose forms were obtained from legal next of kin.

Case Presentations

Case 1

A 63-year-old female patient presented with 2 years of progressive gait difficulties followed by speech changes, then right-sided greater than left-sided weakness. Despite C5-7 diskectomy and fusion for progressive canal stenosis, her weakness worsened. On examination, she had dysarthria, bradykinesia and increased tone with spasticity on the right, and diffuse hyperreflexia. EMG demonstrated positive sharp waves in thoracic paraspinal and gastrocnemius muscles and chronic denervation in right C6-7, L5, and S1 innervated muscles. She was diagnosed with amyotrophic lateral sclerosis (ALS) and died 3 years after symptom onset.

Family history was significant for multiple family members with different neurodegenerative diagnoses including ALS, spinocerebellar ataxia, and FTD (Figure 1A). Clinical genetic testing (2019 ALS sequencing panel with CNV detection, Prevention Genetics) revealed a novel intronic *TBK1* splice variant (*TBK1* c.358+3A>G); this variant was also identified in her symptomatic siblings, but not in the asymptomatic sibling. Her symptomatic sibling had a negative ataxia repeat expansion panel (2019 University of Chicago) and negative *C9orf72* expansion and 450-gene ataxia movement disorders panel (2017 UW Laboratory for Precision Diagnostics).

Pathology

Fresh whole brain weighed 1,421 g; there was mild cortical atrophy. Histologic examination predominantly revealed features of MND, with mild evidence of FTLD. Changes associated with MND were more prominent in upper than lower motor neurons. Primary motor cortex demonstrated marked vacuolation and gliosis. Corticospinal tract pallor was observed, consistent with lateral sclerosis. Inclusions of phosphorylated TDP-43 (pTDP-43) were identified in upper motor neurons (Figure 2A).

FTLD-associated findings were much less pronounced, with only mild rarefaction and gliosis of temporal cortex. Despite minimal cortical histologic changes, there was pTDP-43 pathology involving superior, middle, and inferior temporal gyri, generally limited to layer 2; rare inclusions were identified in the frontal cortex (Figure 2B). The TDP-43 pathology included neuronal inclusions with dense, donut-like morphology encompassing the majority of the cytoplasm with centrally placed nuclei (Figure 2B). We designated the FTLD-TDP-43 subtype as a mix of A and B.[1]

There was significant tau pathology in the temporal lobe, including numerous neurofibrillary tangles and pretangles, predominantly involving layers 2 and 5. A glial tauopathy was also identified, including granular/fuzzy astrocytes scattered throughout all gray matter layers and numerous, slightly granular oligodendroglia coiled bodies in white matter. This pattern is most consistent with argyrophilic grain disease (AGD). Other AGD features included dendritic swellings (so-called grains), especially in the amygdala, scattered astroglial tau with fuzzy or granular appearance, and numerous oligodendroglial coiled bodies (Figure 2D and Table 1; see eAppendix 1 for additional features).

Case 2

A 60-year-old male patient presented with 10 years of progressive cognitive impairment and inappropriate behavior. On examination 2 days before death, he had limited spontaneous speech and was unable to follow most commands and visual cues. He had bilateral first dorsal interossei atrophy and fasciculations in his left upper and lower extremities. Brain MRI was remarkable for profound frontotemporal atrophy. He was diagnosed with FTD-MND.

He had an extensive family history of dementia with psychiatric features (Figure 1B). One sister experienced cognitive decline in her 50s and at autopsy was found to have frontotemporal lobar degeneration. Genetic testing in the proband revealed a premature termination *TBK1* variant (*TBK1* Arg117Ter; 2019 ALS sequencing panel with CNV detection, Prevention Genetics). *C9orf72* expansion testing was negative (2019 Prevention Genetics). No other family members were known to have undergone genetic testing.

Pathology

Fresh whole brain weighed 1,384 g. Grossly, mild cortical atrophy and atrophic anterior nerve roots were noted. Histologic features of MND included mild corticospinal tract pallor, marked loss of anterior horn motor neurons, and occasional Bunina bodies. pTDP-43 inclusions were identified in the motor cortex and in lower motor neurons of the medulla and spinal cord (Figure 2A).

FTLD neurodegenerative change included mild frontotemporal parenchymal vacuolation, predominantly in superficial cortical layers. pTDP-43 pathology was noted most prominently in inferior and middle temporal gyri, with involvement of all cortical layers and subcortical white matter. Granular cytoplasmic pTDP-43 neuronal inclusions in large neurons, often in a perinuclear pattern, and dense cytoplasmic inclusions in small neurons and glia, both in gray and white matter, were observed. There was also granular cortical parenchymal pTDP-43 pathology (Figure 2B). This pattern could be designated as FTLD-TDP type B, but may also fit criteria for the more recently described type E.[2]

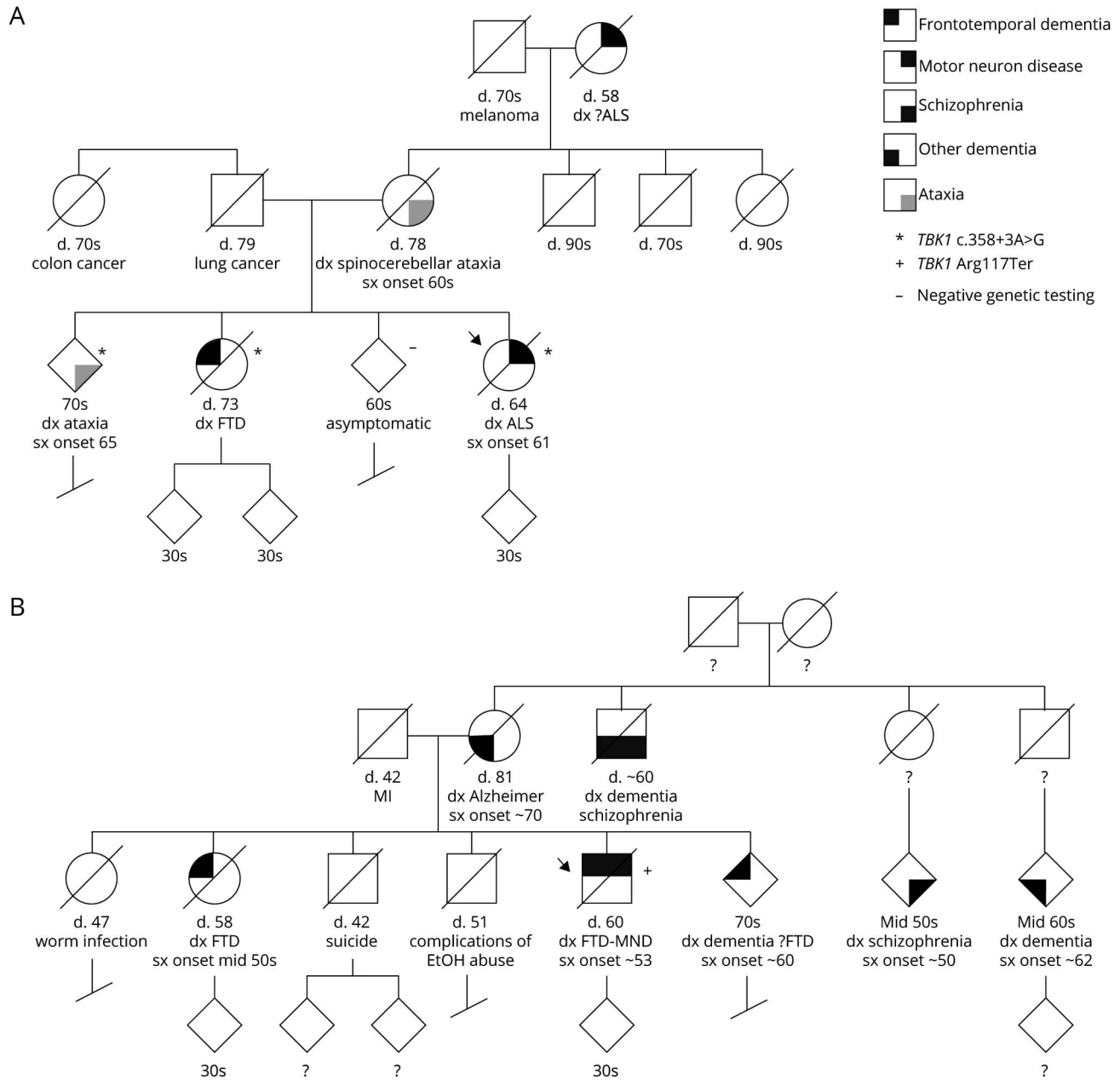
Immunohistochemical staining for pTau revealed features of AGD, similar to those described in case 1 (Figure 2D, Table 1; also eAppendix 1).

Discussion

More than 100 pathogenic variants in *TBK1* have been identified, linked to a broad spectrum of clinical presentations including amnestic dementia, corticobasal syndrome, primary progressive aphasia, primary lateral sclerosis, and spinal muscular atrophy.[3-7] The phenotypic diversity of *TBK1* variants is further illustrated in our 2 families. We postulate that the novel c.358+3A>G variant found in case 1 and her siblings is the same pathogenic variant responsible for her mother's ataxia syndrome and her maternal grandmother's ALS (Figure 1A). Two neighboring nucleotide substitutions, c.358+2T>C and c.358+5G>A, have previously been reported as associated with this phenotypic spectrum[8,9]; we speculate that the variant identified here weakens a splice donor site resulting in *TBK1* haploinsufficiency.

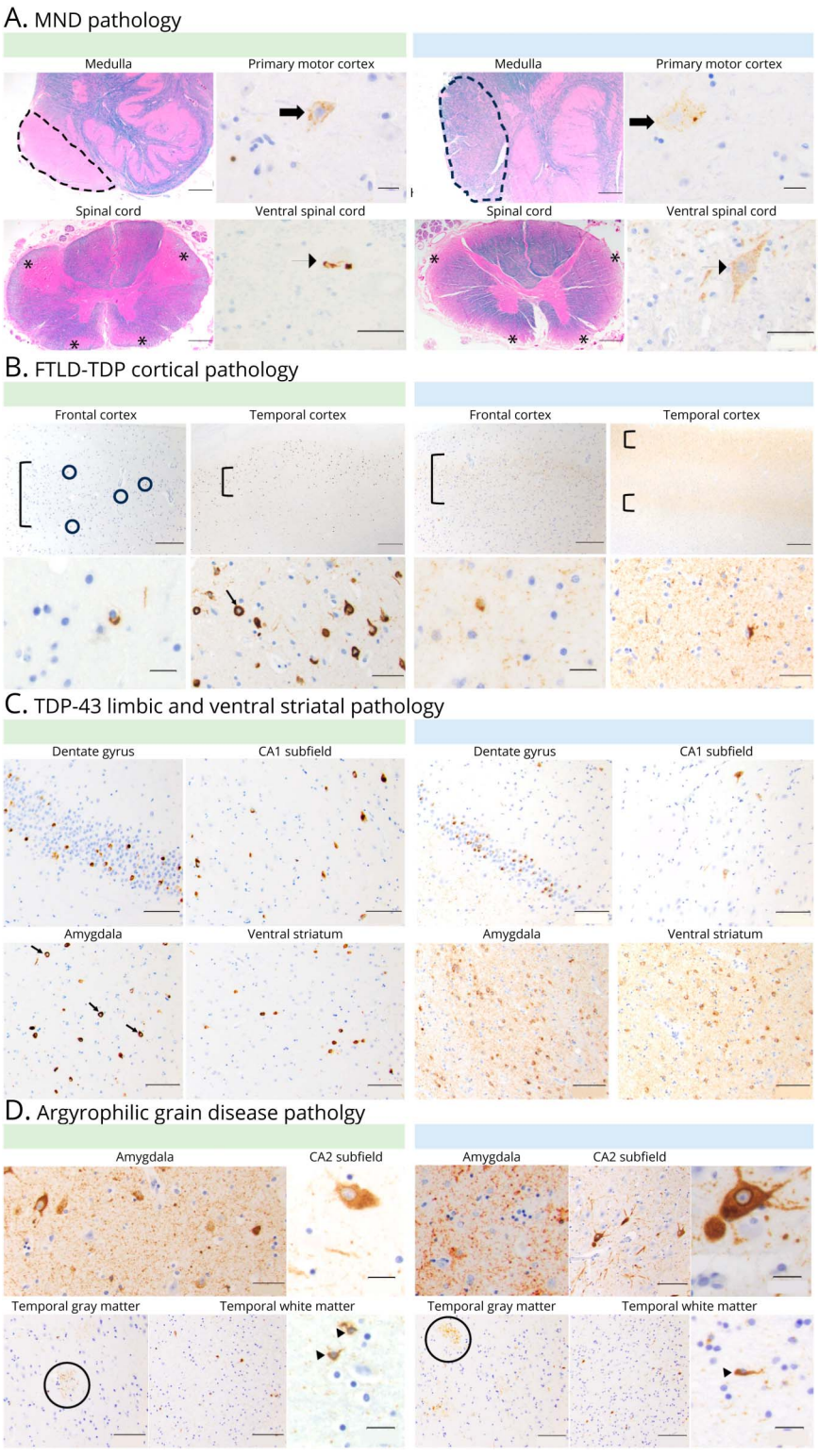
Although all pathogenic *TBK1* variants are associated with TDP-43 pathology, the histologic features are highly variable. In our extensive pathologic evaluation of 2 cases, there were distinct patterns of distribution and inclusion morphology. In case 1, there was a predominance of pathology in upper motor neurons, consistent with the clinical presentation. Although

Figure 1 Family Pedigrees



(A) Family pedigree of case 1, including a sibling and parent with ataxia and a grandparent with presumed amyotrophic lateral sclerosis (ALS). (B) Family pedigree of case 2, including a sibling with autopsy confirmed frontotemporal lobar degeneration. FTD = frontotemporal dementia; MND = motor neuron disease.

Figure 2 Pathologic TDP-43 and Tau Characteristics



(A) *Features of MND*. Case 1 showed prominent pallor of the pyramids in the medulla on H&E/LFB stain, but this was not apparent in case 2 (hash-marked outline). Both cases showed pallor of the corticospinal tracts in sections of the spinal cord (asterisks), but this was more severe in case 1. Both cases showed fibrillar pTDP-43 inclusions in upper motor neurons (arrow). The pTDP-43 pathology of lower motor neurons of the anterior horn of the spinal cord included dense cytoplasmic inclusions in case 1 and granular cytoplasmic inclusions in case 2 (arrowhead). (B) *Features of FTLD-TDP*. Case 1 showed only minimal pTDP-43 pathology in the frontal cortex that was primarily limited to superficial cortical layers and included dense cytoplasm inclusions and rare neurites (circles). The temporal cortex showed more prominent pTDP-43 pathology that included dense cytoplasmic inclusions, some which had donut-like morphology with centrally located nuclei (arrow) and scattered neurites. Although the pathology was more prominent in superficial cortical layers, pTDP-43 pathology was observed in all layers. Case 2 had more severe cortical pTDP-43 pathology in both frontal and temporal cortex compared with case 1. All cortical layers were involved and include both dense and granular cytoplasmic inclusions as well as granular parenchymal pathology. (C) *Features of limbic and ventral striatal pathology*. Both case 1 and case 2 had significant hippocampal pTDP-43 pathology that included dense paranuclear inclusions in the dentate gyrus. Case 1 had more significant CA1 pathology with dense, tangle-like pTDP-43 inclusions while case 2 had more limited CA1 pathology consisting of scattered granular cytoplasmic pTDP-43 inclusions. The amygdala and ventral striatal pTDP-43 had a similar morphologic appearance to the cortical pTDP-43 in each case. For case 1 this included dense, donut-like cytoplasmic inclusions and for case 2 this was primarily granular cytoplasmic and parenchymal pathology. (D) *Features of AGD.* Both case 1 and case 2 had the neuropathologic features of argyrophilic grain disease. This includes dendritic swellings (grains) in the neuropil, as seen in the amygdala; perinuclear accentuation of hyperphosphorylated tau (ptau) in CA2 pyramidal neurons of the hippocampus; fuzzy/granular astrocytes in temporal gray matter (circled); and oligodendroglial coiled bodies in white matter (arrowheads).

she did not have a dementia syndrome, at autopsy there were pathologic changes consistent with FTLD. The second individual exhibited full clinical features of FTD-MND, and the neuropathology was consistent with his clinical presentation, showing pTDP-43 in upper and lower motor neurons and extensive involvement of frontotemporal cortices. Based on current FTLD-TDP consensus classification, case 1 is best classified as a mix of FTLD-TDP type A and B and case 2 as

Table 1 Summary of TDP-43 and Tau Pathologic Findings

	Case 1	Case 2
pTDP-43 distribution	MND: Primary motor cortex is most involved; sparse pathology in spinal cord; no pathology in brainstem FTLD: Temporal >> frontal cortex; layer 2 predominance; minimal white matter pathology; minimal neostriatum pathology	MND: Primary motor cortex, brainstem, and spinal are all moderately involved FTLD: Temporal > frontal cortex; all cortical layers; prominent white matter pathology; moderate brainstem pathology; variable neostriatum pathology (globus pallidus > caudate > putamen)
pTDP-43 morphology	Dense perinuclear donut-like morphology encompassing the majority of the cytoplasm with centrally placed nuclei Scattered short neurites No intranuclear inclusions Skein-like inclusions in motor neurons Rare glial inclusions	Granular cytoplasmic neuronal staining Granular parenchymal staining but minimal neurites Dense Lewy-body like inclusions in pigmented nigral neurons No intranuclear inclusions Numerous glial inclusions
FTLD-type	FTLD-TDP-43 Type A/B	FTLD-TDP-43 Type B or Type E
ADNC	Low (Thal 2, Braak precluded, CERAD absent)	Low (Thal 2, Braak IV, CERAD absent)
Lewy body pathology	Not identified	Not identified
Tau pathology	Features of argyrophilic grain disease - Oligodendroglial coiled bodies - Grains - Perinuclear ptau accentuation in CA2 hippocampal neurons	

Abbreviations: ADNC = Alzheimer disease neuropathologic change; FTLD = frontotemporal lobar degeneration; MND = motor neuron disease.

type B or type E.[1,2] There have been some suggestions that type E may exist on a continuum with type B, potentially representing an earlier pathologic stage.[10]

These 2 cases support potential association between *TBK1*-related TDP-43 and tau aggregates, specifically argyrophilic grain disease (AGD). Although AGD is considered an age-related proteinopathy, it does occur in people <65 years of age. There is an age-related increase in prevalence, ranging from about 9.3% in patients age 65 years to 31.3% in centenarians.[11] However, a study of early-onset Alzheimer disease (AD) demonstrated AGD prevalence up to 41%, higher than in a general aging cohort, but still lower than late-onset AD with a prevalence of 58%.[12] Therefore, in our cases, given the younger age and lack of significant comorbid AD neuropathologic change, the presence of AGD is somewhat unexpected (see eAppendix). It cannot be determined based on observations of postmortem tissue alone whether there is a causal link; however, there are other reported cases of *TBK1* pathogenic variants with comorbid AGD.[7,13-16]

TDP-43 and tau copathologies have been observed in non-*TBK1* FTLD-TDP and FTLD-MND. A potential specific link between *TBK1* and tau pathology could lie in the dysfunctional TBK1 protein, which may affect tau turnover by autophagy. It is hoped that future comprehensive clinicopathologic characterization of *TBK1* families will help elucidate the exact mechanisms responsible for varying clinical phenotypes and neuropathologic findings.

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Kimiko Domoto-Reilly, MD, MMSc	Department of Neurology, University of Washington	Drafting/revision of the manuscript for content, including medical writing for content; study concept or design; analysis or interpretation of data
B. Jane Distad, MD	Department of Neurology, University of Washington	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data; study concept or design

Continued

Appendix *(continued)*

Name	Location	Contribution
Danny E. Miller, MD, PhD	Department of Laboratory Medicine and Pathology; Department of Pediatrics; Brotman Baty Institute for Precision Medicine, University of Washington	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data
Yi-Han Lin, MD	Department of Neurology, University of Washington	Drafting/revision of the manuscript for content, including medical writing for content; major role in the acquisition of data
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Andrew S. Warren, BA, BS	College of Osteopathic Medicine, Pacific Northwest University of Health Sciences	Drafting/revision of the manuscript for content, including medical writing for content
Suman Jayadev, MD	Department of Neurology; Department of Laboratory Medicine and Pathology; Department of Medical Genetics, University of Washington	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
Caitlin S. Latimer, MD, PhD	Department of Laboratory Medicine and Pathology, University of Washington	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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