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BRIEF COMMUNICATION

Novel *NUDT2* variant causes intellectual disability and polyneuropathy

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Abstract

Exome or genome sequencing was performed to identify the genetic etiology for the clinical presentation of global developmental delay, intellectual disability, and sensorimotor neuropathy with associated distal weakness in two unrelated families. A homozygous frameshift variant c.186delA (p.A63Qfs*3) in the *NUDT2* gene was identified in cases 1 and 2 from one family and a third case from another family. Variants in *NUDT2* were previously shown to cause intellectual disability, but here we expand the phenotype by demonstrating its association with distal upper and lower extremity weakness due to a sensorimotor polyneuropathy with demyelinating and/or axonal features.

Introduction

NUDT2 is located on chromosome 9 at position 9p13.3 and encodes a protein of 147 amino acids.[1] *NUDT2* encodes for Nudix hydroxylase 2, which belongs to the Nudix (nucleoside diphosphate linked to x) hydrolase superfamily. As a diadenosine tetraphosphate (AP_4A) hydrolase,[2] it is thought to play an important role in a number of cellular processes, including the metabolism of toxic byproducts, DNA replication, and cell proliferation, among others,[3,4] and may be a prognostic marker in breast carcinomas.[3] *NUDT2* has been previously associated with intellectual disability in a small number of families,[5,6] suggesting a role in central nervous system development, but no other neurological presentations associated with this gene have been reported. We report

three cases from two unrelated families with a novel mutation in *NUDT2* that presented with global developmental delay and intellectual disability, but also with length-dependent sensorimotor polyneuropathies with demyelinating and/or axonal features, expanding the phenotype associated with this gene.

Patients and Methods

Cases 1 and 2 are siblings that presented to the neuromuscular clinic at Olive View-UCLA Medical Center, and were subsequently enrolled in the Undiagnosed Diseases Network (UDN)[7] and evaluated at the UCLA Clinical Site. Case 3 presented to Ochsner Children's Health Center. Genetic counseling was provided for all patients prior to and following completion of the study. All patients provided written informed consent. All methods were approved by the National Human Genome Research Institute (NHGRI) central Institutional Review Board.

For cases 1 and 2, whole-genome sequencing was performed on genomic DNA extracted from whole blood samples at the Hudson-alpha Institute UDN sequencing core on the Illumina HiSeq X system generating 150bp paired-end read at the average depth of coverage of >50X. Coverage at the flanking bases of the variant position was as follows: 29x (proband), 84x (father), 80x (mother), 46x (sister). Data analysis was performed at UCLA using a custom-developed pipeline as described previously.[8] For case 3, using genomic DNA extracted from whole blood from the proband and buccal samples from the parents, the exonic regions and flanking splice junctions of the genome were captured using the IDT xGen Exome Research Panel v1.0. Massively parallel (NextGen) sequencing was performed on an Illumina system with 100bp or greater paired-end reads at an average depth of coverage of 77.7X. Reads were aligned to human genome build GRCh37/UCSC hg19, and analyzed for sequence variants using a custom-developed analysis tool. Additional sequencing technology and variant interpretation protocol have been previously described.[9] The general assertion criteria for variant classification are publicly available on the GeneDx ClinVar submission page (http://www.ncbi.nlm.nih.gov/clinvar/submitters/26957/).

Results

Case reports

Case 1

A 23-year-old male presented with global developmental delay, distal upper and lower extremity weakness, and cognitive impairment (Table 1). He was born full term without perinatal or postpartum complications, but the mother lost his twin pregnancy at 10 weeks of gestation. He walked at the age of 3 and was unable to speak intelligibly until 6 years of age. During adolescence he developed gait difficulties and frequent falls that progressively worsened. The family also reported behavioral issues. He required assistance with activities of daily living due to physical limitations. Family history was significant for a younger sister with similar symptoms, reported as Case #2.

On examination he was fully oriented. His speech was non-fluent with simple word sentences. He had bilateral ptosis (Fig. 1A), slow tongue movements, and normal muscle tone with atrophy in the distal lower extremities. Strength was decreased in distal upper and lower extremities. Sensation to pinprick was decreased distally in all extremities. Vibration and proprioception were diminished in the distal lower extremities and reflexes were absent. He had intention tremors and a steppage gait with overpronation.

Neuropsychological testing demonstrated impaired general intelligence (FSIQ = 60). Reading ability was at the third-grade level, and performance on nonverbal tasks was generally better than on verbal tasks. Cognitive impairments were found in most domains (attention/processing speed, language, visuospatial functioning, and executive function), however, memory was largely intact.

Magnetic resonance imaging (MRI) of the brain and cervical, thoracic, and lumbar spine and an electroencephalogram (EEG) were normal. Nerve conduction studies (NCSs) and electromyography (EMG) were consistent with a severe length-dependent sensorimotor polyneuropathy with both demyelinating and axonal features. The conduction velocities (m/s) in the upper extremities were in the high 20s to 30s and the EMG demonstrated acute denervation and chronic denervation/reinnervation in a length-dependent manner.

A sural nerve biopsy demonstrated large and small myelinated fiber loss consistent with a chronic neuropathy. Muscle biopsy of the vastus lateralis demonstrated mild denervation atrophy.

A chromosomal microarray was normal and the initial targeted genetic work up was negative. It included connexin 32, duplication and deletion of peripheral myelin protein 22, Tay-Sachs, Krabbe disease, metachromatic leukodystrophy, X linked adrenoleukodystrophy, and Fragile X syndrome. A hereditary neuropathy panel with 83 genes was performed in 2019, yielding no pathogenic variants. The family was enrolled in UDN and whole genome sequencing was performed to identify a homozygous frameshift variant in *NUDT2* NM_001244390.1:c.186del (p.(Ala63GlnfsTer3)). The patient's affected sister, case 2, was also found to have the same homozygous variant. Both parents were heterozygous carriers, were asymptomatic, and lacked any dysmorphic features.

Table 1. *NUDT2* pathogenic variants reported to date and associated phenotypes.

Patients	Cases #1 and #2 (siblings)	Case #3	Cases #4 and 5* (siblings)	Cases #6-10** (3 unrelated families)
Variant	NM_001244390.1:c.186del (p.(Ala63GlnfsTer3))	NM_001244390.1:c.186del (p.(Ala63GlnfsTer3))	NM_001161.5 c.34C> T (p.Arg12)***	NM_001244390.1:c.34C> T (p. Arg12*)
Development and Dysmorphic features	Global developmental delay, bitemporal narrowing, midface hypoplasia, bilateral ptosis, high arched palate. Case 1 also with retrognathia and left eye exotropia	Global developmental delay, slightly upslanting palpebral fissures, broad nasal tip, short flat philtrum, and overfolded helices	Global developmental delay Subtle facial dysmorphisms Weak sucking in infancy Hypotonia	Global developmental delay Subtle facial dysmorphisms Weak sucking in infancy Hypotonia Low birth weight and height Microcephaly
Motor	Falls, distal upper and lower extremity weakness	Distal upper and lower extremity weakness	Falls, unsteady gait	Not reported
Neuropathy and Conduction Velocity	Length-dependent sensorimotor with demyelinating (28-34m/s in upper extremities) and axonal features in #1, axonal length-dependent sensorimotor in #2 (41-44 m/s)	Axonal, length-dependent, sensorimotor polyneuropathy (40-50s m/s)	Not reported	Not reported
Language	Slow speech Frequent one-word responses	Dysarthria Multi-word sentences	Dysarthria	Not reported
Cognition	Mild intellectual disability in Case 1. Case 2 did not undergo formal neuropsychological testing.	Formal neuropsychological testing not performed	Intellectual disability	Borderline normal intelligence
Progressive	Yes	Yes	No	No
Brain MRI	Normal in #1 Not performed in #2	Normal	Possible iron deposition in globus pallidus bilaterally	One patient with findings suggestive of partially impaired myelination, thinning of corpus collosum

*As reported by Anazi et. al.[5]
**As reported by Yavuz et. al.[6]
***Canonical transcript ID.

Case 2

A 10-year-old girl, sister of case 1, presented with global developmental delay, weakness and falls (Table 1). She was born at full term without complications. She did not walk until 2.5 years of age and began using single words at age 2.

On examination the patient was attentive but oriented only to self. Speech was dysarthric with impaired repetition. She had facial weakness (Fig. 1B), normal tone, and decreased muscle bulk and weakness distally in all extremities. Vibratory sensation was decreased in the distal lower extremities. Reflexes were absent and she had a mild steppage gait with overpronation.

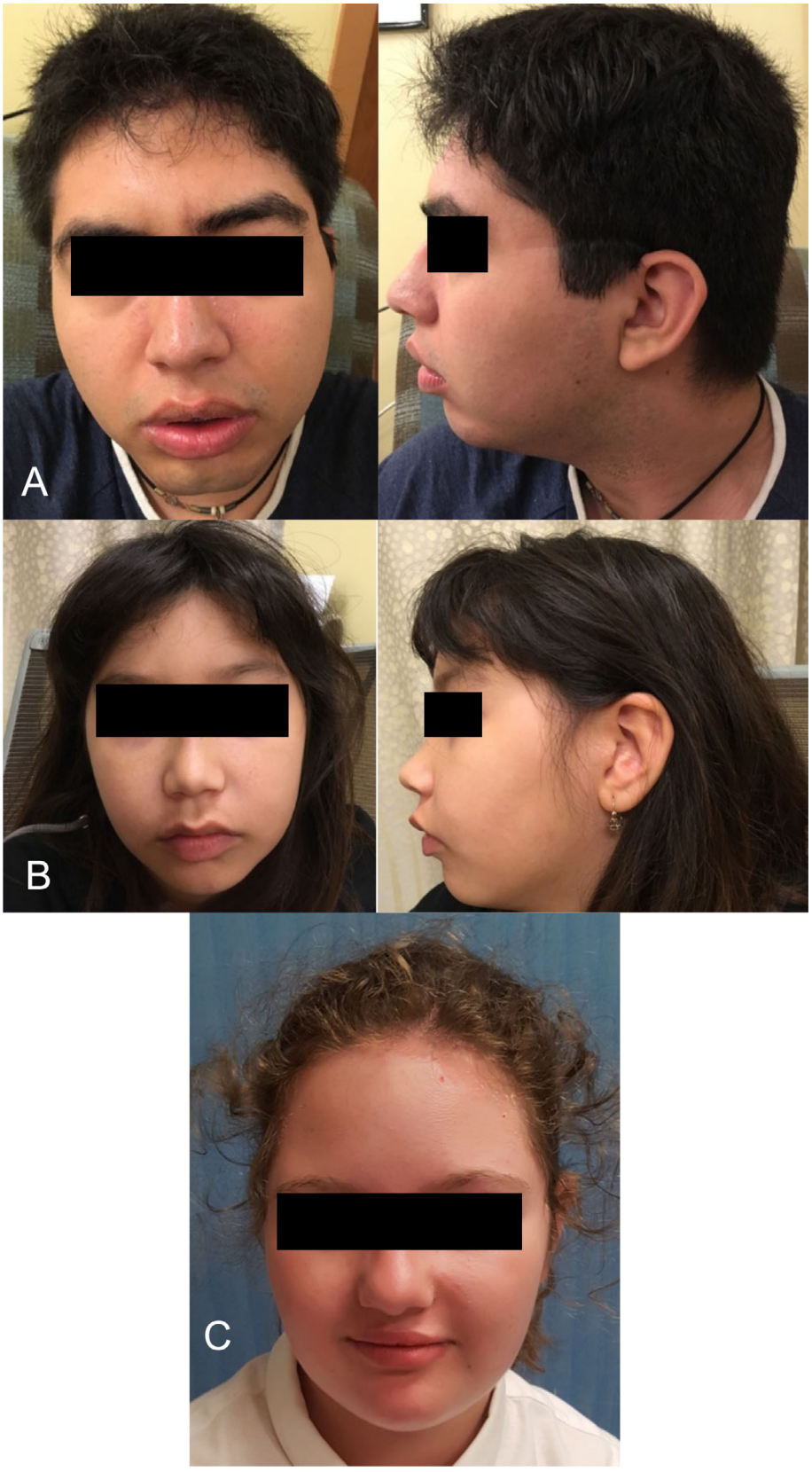
NCSs demonstrated absent sural responses, absent or reduced CMAP amplitudes in the lower extremities, and length-dependent acute and chronic denervation on EMG. Overall, the findings were consistent with a severe length-dependent axonal sensorimotor polyneuropathy.

On genetic testing she was found to have the identical homozygous frameshift variant in NM_001244390.1: c.186del (p.(Ala63GlnfsTer3)) as her brother/Case #1.

Case 3

A 12-year-old girl (Fig. 1C) presented with developmental delays, acquiring speech and ambulation at 2 years of age (Table 1). She was born to non-consanguineous parents

Figure 1. Patient facial features. (A) Patient 1 demonstrated bitemporal narrowing, midface hypoplasia, bilateral ptosis, left eye exotropia, high arched palate, and retrognathia. (B) Patient 2 demonstrated bitemporal narrowing, midface hypoplasia, bilateral ptosis, and high arched palate. (C) Patient 3 demonstrated slightly upslanting palpebral fissures, broad nasal tip, short flat philtrum, and overfolded helices. Parental consent for publication of these photographs was obtained.



at 37 weeks by C-section. Prenatal history was significant for gestational diabetes.

On examination she was fully oriented. Speech was dysarthric with normal cranial nerves but with decreased muscle bulk in the lower extremities. Strength was decreased in wrist flexors and extensors, finger flexors and extensors, hip flexors, ankle flexors and extensors. Sensation was intact to light touch. Reflexes were decreased in the upper extremities and absent in the lower extremities. A mild intention tremor was noted. Basic laboratory examination and MRIs of the brain and spine were normal.

NCS and EMG showed evidence of an axonal sensorimotor neuropathy. The SNAP amplitudes in the lower extremities were significantly reduced but the upper extremity was normal except for the radial sensory response. EMG of the lower extremities demonstrated acute denervation and chronic denervation/reinnervation in a length-dependent manner.

Genetic testing was negative for fragile X syndrome and Friedreich ataxia. A single-nucleotide polymorphism (SNP) array was negative for copy number variation but showed 4 regions of homozygosity (ROH). Exome sequencing identified the identical homozygous *NUDT2* variant, NM_001244390.1:c.186del (p.(Ala63GlnfsTer3)) reported above and it was noted to be within a region of homozygosity (ROH) that was detected by microarray. Both parents were found to be heterozygous carriers, were asymptomatic, and lacked any dysmorphic features.

Discussion

We report three patients from two families with sensorimotor polyneuropathy and intellectual disability due to a newly described pathogenic variant in *NUDT2*. While one founder variant in *NUDT2* has been reported in cases with intellectual disability,[5,6] the three cases presented here also developed profound neuropathies resulting in distal weakness in addition to intellectual disability.

The previous seven cases described with intellectual disability were of Saudi Arabian origin and were found to have a different founder variant in *NUDT2*, homozygous nonsense variant NM_001244390.1:c.34C>T (p.Arg12*).[5,6] The first study described the variant in two cases with intellectual disability, hypotonia, and unsteady gait (Table 1). The second study[6] described five patients from three unrelated families that shared delayed motor and language development, and borderline intelligence, among other features (Table 1) The variant presented here does not appear to be a founder mutation. Cases 1 and 2 are from Mexican descent while Case 3 is of Cajun descent. Although unknown consanguinity is a possibility for case 3, cases 1 and 2 did not share any ROH on chromosomal microarray. In addition the prevalence of this variant among non-Finnish Europeans and Latinos in gnomAD seems consistent with an autosomal recessive allele at 0.02% and 0.0086%, respectively.

Only 10 patients with neurological symptoms due to *NUDT2* mutations have been described. Thus, our knowledge regarding the physiological and pathophysiological role of *NUDT2* is limited. However, *NUDT2* has been associated with intellectual disability in all the cases reported to date, and sensorimotor polyneuropathy in the three cases presented here. We do not know if the slowly progressive polyneuropathy we observed is invariably present or specific to certain *NUDT2* mutations because it appears that nerve conduction studies were not performed in previous cases. The mechanistic reason for the presence of progressive polyneuropathy is unclear. We hypothesize that the truncated protein produced has a toxic cellular effect in peripheral nerves. Since the mutation is in the last exon, it is unlikely to undergo nonsense mediated decay(NMD),[10] and our RNAseq data observed many reads with the deletion, arguing against this mechanism (data not shown). However, more research on NUDT2 function in the central and peripheral nervous system, and further clarification of the genotype-phenotype correlations of this variant are needed. Such correlations should become evident as more cases are identified with more widespread use of exome and genome sequencing.[11] We recommend that mutation of *NUDT2* be considered in patients with otherwise unexplained intellectual disability and sensorimotor polyneuropathy.

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Conflicts of Interest

Michelle Morrow and Richard Person are employees of GeneDx, Inc., where the data from Case 3 was analyzed.

Author Contributions

F.D. and S.K. contributed to data acquisition and analysis and drafted a significant portion of the manuscript. D.N., H.L, R.P., M.M., R.S., N.D., A.Z., M.H., R.F., J.B.B., and Y.C. contributed to data acquisition and analysis. J.A.M., C.P., and S.F.N. supervised the study and contributed to data acquisition. B.L.F. and S.K.M. contributed with conception and design of the study, data acquisition and analysis, supervised the study, and drafted a significant portion of the manuscript. All authors performed a critical review of the manuscript. Please refer to the

Supplementary Table for a listing of the Undiagnosed Diseases Network members and affiliations.

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Supporting Information

Additional supporting information may be found online in the Supporting Information section at the end of the article.

Supplementary Material. Undiagnosed Diseases Network members and affiliations.

