Novel Copy Number Deletion involving NUS1 associated with Epilepsy, Tremor, and Intellectual Disability

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Abstract

Copy number variations (CNVs), among other genetic abnormalities, have been implicated in a range of disorders and can result in a variety of clinical manifestations, such as intellectual disability, developmental disorders, and cancer. The role of specific genes within such CNVs, especially novel or rare genes, is the subject of ever-advancing contemporary genetics research. The identification and characterization of these genes play an important role in understanding the pathophysiology of many different medical conditions and shed light on diagnosis refinement and different strategies for treatment. This case study looks at...
the genetics underlying the clinical presentation in a patient exhibiting epilepsy, spinal abnormalities, and intellectual disability, with a focus on identifying the underlying genetic basis for this presentation. Here we show a novel de novo 2.62 Mb interstitial deletion at 6q22.1-q22.31 implicates the NUS1 gene as a significant contributor to the observed phenotypes. NUS1 classically has been associated with congenital disorders of glycosylation and intellectual disability. However, our findings regarding this deletion suggest a broader phenotypic spectrum for NUS1 variants, encompassing manifestations like kyphosis, choreoathetosis, and possible dyskinesias, which were not notably linked to this gene before. This paints a clearer picture of NUS1’s clinical relevance beyond previously known links. The results of this report also add to the building body of evidence that highlights the significance of CNVs in neurological and developmental disorders. Our findings reinforce the importance of genetic analysis in patients presenting with atypical symptoms.

CASE PRESENTATION

Clinical Presentation and Family History

The subject is a 33-year-old man presenting with mild-to-moderate intellectual disabilities; the family has spent a lifetime on an odyssey to understand the subject’s condition and to collaborate with clinicians to develop a best treatment strategy. The subject has mild facial dysmorphology that includes large ears. He also has excessive drooling. He has scoliosis, gait disturbance, slender build, slender fingers, and a history of extensive tremors. He was born to a non-consanguineous family. He has a healthy and neurotypical younger male sibling. He presents with global developmental delays, attention deficit disorder, hyperactivity, impairment of fine and gross motor coordination, generalized hypotonia, and temper tantrums. Anxiety, impulsivity, and depression were also reported and he was diagnosed with anxiety and mood arousal regulation disorder in 2007. Neuropsychological evaluation was remarkable for poor attention span. Family history is noted for leukemia in his paternal grandfather and possibly autism in his father’s sister’s son. There is a history of autism spectrum disorder in both the paternal and maternal side of the family.

The proband was born first in birth order from an unremarkable and uncomplicated pregnancy, other than light spotting in the first trimester. He was born at full term via normal, spontaneous vaginal delivery with a birth weight of 8 lbs. 4 1/2 oz (95th percentile). There were no perinatal complications. The proband’s first febrile seizure was noted at 11-12 months of age. The seizure was mild and lasted ten minutes in duration. He then had multiple seizures after 17 months of age. Recurrent seizures consist of blank stares, and repetitive jerks of the head without dropping, that lasted a few seconds in duration. These episodes were very frequent and may occur 10 to 20 times over a period of thirty minutes. Ethosuximide was not effective in treating seizures and was replaced with valproic acid with an improved response noted.

He had a history of no expressive language and an attention deficit disorder. During an early clinic visit as a child, the boy was observed to be social but nonverbal. He had absent speech but could communicate using simple signing. EEG and CT scans were first conducted at one year of age and were both unremarkable. Analyses indicated that the critical DNA region for AS had not been deleted; although it was noted that additional DNA markers were needed to completely rule out AS.

At 3 years, 3 months of age, referral to medical genetics was made. The proband’s height, weight, and head circumference were at the 85th, 75th, and 45th percentile, respectively. The neurological exam showed hyperactivity, impairment of fine and gross motor coordination, and generalized hypotonia. Neuropsychological evaluation was remarkable for poor attention span and learning and intellectual disability. His expressive language was assessed as being less than an eight month level and his receptive language was at 2 to 2 1/2 year level. A Test of Nonverbal Intelligence Test (TONI-4) was administered twice to assess abstract reasoning and problem solving, of which he scored 85 and 119, respectively on an index score of 100 and a standard deviation of 15. At the same time, cytogenetic evaluation was performed to rule out Angelman syndrome, (AS). Analyses indicated that the critical DNA region for AS had not been deleted; although it was noted that additional DNA markers were needed to completely rule out AS.

At four years of age, he had a weight, height, and head circumference of 50th, 90th, and 45th percentile, respectively. His weight percentile dropped from 85th to 50th, and to this day he is lean. He did not have
expressive language and could only say “hi” or “bye.” The subject was able to follow one and two-step commands and had poor coordination. The proband was referred to a psycholinguistic evaluation and was diagnosed with Seizure disorder and Developmental Receptive and Expressive Language disorder. At six years of age, EEG was indicative of diffuse cerebral dysfunction, and consistent with a diagnosis of primary generalized epilepsy. He only developed limited spoken language despite receiving nonverbal cognitive scores in the average range of his age. Valproic acid was increased to 500 mg bid, from 125 mg bid and carnitine was recommended. By 10 years of age, the proband’s nonverbal mental age did not continue to maintain the same rate of change as his physical age, dropping to an IQ of 68, as measured by the Leiter-R. He also underwent back surgery that year for correction of severe kyphosis.

The next follow-up psychiatric visit was when the subject was 17 years of age. He was noted to be ambulatory with abnormal gait, dysarthric, with a continuance of tremors and seizures. Due to his significant hand tremors, he required assistance with his daily living routine. He did not exhibit self-injurious behavior and psychosis was not observed.

The proband was seen again for neuropsychological evaluation at 21 years of age. His general intelligence function was assessed with the Stanford Binet Intelligence Scales, Fifth Edition. He obtained a Verbal IQ of 43 (<1st percentile), a Nonverbal IQ of 52 (<1st percentile), and a combined IQ of 45 (<1st percentile). This score placed him in the moderately delayed range of general intelligence function. His adaptive behavior was also estimated with the Vineland Adaptive Behavioral Scales, Second Edition, which placed him at an approximate age equivalent of 3 years 7 months. His adaptive skills, including communication, daily living skills, and socialization, were hindered in part by delays in communication skills and significant tremors.

In 2023, and at 31 years of age, he returned again to the George A. Jervis Clinic at the New York Institute for Basic Research, Staten Island, New York for an updated psychological and genetic evaluation. The proband continued to have extensive tremors which affected his daily functioning such as feeding himself. He also had choreoathetosis and possible dyskinesias. Ataxia was not noted. At the visit, Fragile X testing was reported as negative. Brain MRI was unattainable because of the steel rods placed following kyphosis correction surgery. The proband’s lipid panel was obtained (Table 1) and showed abnormally elevated levels of total cholesterol, triglycerides, LDL cholesterol, and low HDL cholesterol, but a normal BMI of 21.7 kg/m². In 2023, additional lab testing did not find any abnormality with his oxysterol profile (see Table 2). Table S1 shows a comprehensive list of the current medications of the proband. Epilepsy is currently controlled with clonazepam.

Genomic Analyses

A whole genome single nucleotide polymorphism (SNP) microarray was performed as a trio on the subject, mother, and father. A de novo 2.62 Mb interstitial deletion of 6q22.1q22.31 was detected, which is interpreted as pathogenic, as seen in Table 3. This interval includes nine OMIM genes (VGLL2, ROS1, GOPC, NUS1, CEP85L, PLN, MCM9, ASCH1, MAN1A1). In addition, the microarray also identified a 1.16 mb interstitial duplication of 7p21.2, p21.1 and 77 kb interstitial duplication of 22q12.1q12.1 in the proband. This interval includes 6 OMIM genes (BZW2, TSPAN13, AGR2, AGR3, AHR, SNX13). At this time, no clinically established disorders have been reported with duplication of this region.

DISCUSSION

The NUS1 (nuclear undecaprenyl pyrophosphate synthase 1) gene encodes the Nogo-B receptor (NgBR), a subunit that is essential protein for dolichol synthesis. Dolichol serves as a carrier for the oligosaccharide chain of N-linked glycosylation, which contributes to proper folding and quality control of proteins. (Chiu et al. 2007). In addition to cellular dolichol synthesis and protein glycosylation, NgBR is responsible for lysosomal cholesterol accumulation. Specifically, lysosomal cholesterol accumulation contributes to movement deficits associated with NUS1 haploinsufficiency (Yu et al. 2021). NUS1 was among many candidate genes that were found to be associated with developmental and epileptic encephalopathy (DEE), a group of conditions characterized by co-occurrence of epilepsy and intellectual disability (Hamdan et al. 2017). A previous study reported six individuals with seizures and overlapping microdeletion on 6q22.1q22.31, narrowing the critical
region to 250 kb. This region encompasses NUS1 and the promoter of SLC35F1, which were important contributors to tremors and epilepsy (Szafranski et al. 2015). Variants of NUS1 have also been linked with ataxia (Den et al. 2019). In some less common cases, NUS1 contributes to parkinsonism, scoliosis, gestational diabetes mellitus, and psychosis (Den et al. 2019; Jiang et al. 2022; Fraiman et al. 2021). NUS1 is classically associated with Congenital Disorder of Glycosylation, Type Iaa, and autosomal dominant type 55 intellectual disability with seizures (Hamdan et al. 2017; Yu et al. 2021). So far, there have been reports of nine de novo NUS1 variants linked to developmental and epileptic encephalopathy (Den, 2019). Individuals with congenital disorders of glycosylation are susceptible to a variety of different symptoms across several different organ systems, which include developmental delays, poor growth, nerve damage, endocrine dysfunction, and facial dysmorphism. There are over 130 different diseases linked to dysfunctions in the glycosylation pathway, many of which do not have any treatment (Chang, He, and Lam 2018). Although some congenital disorders of glycosylation can be found to have elevated oxysterols (Dang Do et al. 2023), this did not appear to be the case in our patients, perhaps suggesting that the intact copy of NUS1 on the other chromosome is sufficient to prevent substantial oxysterol accumulation.

ASF1A is a gene responsible for encoding a histone chaperone protein belonging to the H3/H4 family and regulates nucleosome assembly (Liang et al. 2017). All three studies reported in Clinvar that involve ASF1A deletion have a variant length over 1kb involving multiple genes, making it difficult to attribute the connection between ASF1A to the neurodevelopmental symptoms. VGLL2 is a gene that encodes for the transcription factor of vestigial-like protein 2 that is associated with skeletal muscle development (Hitachi et al. 2019). Variants including deletions in this gene had other genes deleted including NUS1 (Szafranski et al. 2015). Thus, the precise contribution of this gene or its lack thereof could not be determined. Similarly, GOPC is a gene that encodes a Golgi protein that could contribute to infertility (Bizkarguena et al. 2019). Variants in ClinVar included NUS1 and other genes, making it difficult to determine its contribution. Finally, ROS1, a proto-oncogene that encodes for either a protein or differentiation factor could contribute to tumor development (Drilon et al. 2021). Additional variants with ROS1 deletion included NUS1 and other genes and did show phenotypes of delayed speech and language development as well as intellectual disability. Thus, based on our search, the patient’s phenotype of seizures, tremors, intellectual disability, and delayed speech and language development seem to be more associated with a NUS1 mutation as opposed to the other OMIM genes.

METHODS

Fragile X testing:
Genomic DNA was isolated and CGG repeat primed PCR analysis was performed to determine the fragile X CGG repeat size.

Chromosomal Microarray:
The SNP microarray analysis utilized the Cytoscan HD platform, incorporating over 743,000 SNP probes and 1,953,000 NPCN probes, with a median spacing of 0.88 kb. Genomic DNA from the provided sample type underwent extraction and digestion with NSP1, followed by ligation to NSP1 adaptors. PCR products were subsequently purified and quantified. The purified DNA was fragmented, biotin-labeled, and then hybridized to the Cytoscan HD GeneChip. Data analysis was conducted using the Chromosome Analysis Suite, referencing the GRCh37/hg19 assembly. The test’s development and performance characteristics were established by LabCorp. Microarray analyses were conducted for both the subject and their parents.

Oxysterol Testing:
Assay for oxysterols was performed at Mayo Clinic Laboratories (Test ID#OXYWB). This test was developed and its performance characteristics determined by Mayo Clinic in a manner consistent with CLIA requirements. It has not been cleared or approved by the US Food and Drug Administration.

EEG Testing:
EEG was used to evaluate a seizure disorder and the subject is currently treated with Depakote. The EEG examination was performed while the subject was in three stages of awake, drowsy, and asleep, using the 10-20 system of electrode displacement. Photic stimulation included a stepwise manner from 1-30 Hz.

**General Intellectual Function and Adaptive Function:**

General intellectual function was assessed with the Stanford Binet Intelligence Scale, Fifth Edition. Adaptive function was estimated based on the information the mother provided via phone using the Vineland Adaptive Behavioral Scales, Second and Third Editions.

**Psycholinguistic Evaluation:**

Evaluation methods included direct observation, record review, videotape analysis, parent interview, and the Vineland Adaptive Behavioral Scale, Second Edition. Additional methods to evaluate early language development included the sentence verification task and the Peabody Picture Vocabulary Test. The parents were administered the Vineland Adaptive Behavioral Scale. Unfortunately, the subject was not compliant to participate in this test.

Test of Early Language Development-Second Edition is a standardized psycholinguistic instrument which measures language ability between the ages of newborn and eight years of age. The Sentence Verification Task evaluated comprehension of children with limited productive skills by presenting sentences orally and asking for the judgment of the truth of the sentence. The Peabody Picture Vocabulary test is a standardized psycholinguistic instrument which presents four pictures and asks the child to identify one from the group.

**ADDITIONAL INFORMATION**

**Ethics Statement**

Subject consent was obtained for research and publication, with approval of protocol #7659 for the Principal Investigator: G.J.L., of the George A. Jervis Clinic by the New York State Psychiatric Institute—Columbia University Department of Psychiatry Institutional Review Board.

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**Author Contributions**

J.Y.H, D.H.I., R.A. all contributed to data analysis and preparing the original draft. E.M., M.G. and K.A. assisted with data acquisition. G.J.L. was responsible for project conception, data acquisition, supervising, reviewing, and editing the manuscript.

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**References:**


Jiang, Li, Jun-Pu Mei, Yu-Wen Zhao, Rui Zhang, Hong-Xu Pan, Yang Yang, Qi-Ying Sun, et al. 2022. “Low-Frequency and Rare Coding Variants of NUS1 Contribute to Susceptibility and Phenotype of Parkinson’s Disease.” Neurobiology of Aging 110 (February): 106–12.


Liang, Xiuming, Xiaotian Yuan, Jingya Yu, Yujiao Wu, Kailin Li, Chao Sun, Shuyan Li, et al. 2017. “Histone Chaperone ASF1A Predicts Poor Outcomes for Patients with Gastrointestinal Cancer and Drives Cancer Progression by Stimulating Transcription of β-Catenin Target Genes.” EBioMedicine 21 (July): 104–16.


**Table 1:** Lipid Panel

<table>
<thead>
<tr>
<th>Lipid Panel</th>
<th>Value (High/Low)</th>
<th>Reference Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cholesterol, Total</td>
<td>252 (H)</td>
<td>&lt;200 mg/dL</td>
</tr>
<tr>
<td>HDL Cholesterol</td>
<td>35 (L)</td>
<td>&gt; OR= 40 mg/dL</td>
</tr>
<tr>
<td>Triglycerides</td>
<td>272 (H)</td>
<td>&lt;150 mg/dL</td>
</tr>
<tr>
<td>LDL Cholesterol</td>
<td>172 (H)</td>
<td>&lt;100 mg/dL</td>
</tr>
<tr>
<td>CHOL/HDL Ratio</td>
<td>7.2 (H)</td>
<td>&lt;5.0</td>
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<tr>
<td>Non HDL Cholesterol</td>
<td>217 (H)</td>
<td>&lt;130 mg/dL</td>
</tr>
</tbody>
</table>

**Table 2:** Oxysterols

<table>
<thead>
<tr>
<th>Oxysterols</th>
<th>Concentration (nmol/m)</th>
<th>Reference Value (nmol/m)</th>
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</thead>
<tbody>
<tr>
<td>Cholestane-3β,5α,6γ-triol</td>
<td>&lt; 0.055</td>
<td>[?] 0.070</td>
</tr>
<tr>
<td>7-Ketocholesterol</td>
<td>&lt; 0.038</td>
<td>[?] 0.100</td>
</tr>
<tr>
<td>Lyso-sphingomyelin</td>
<td>&lt; 0.006</td>
<td>[?] 0.100</td>
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**Table 3:** NUS1 Variant Finding

<table>
<thead>
<tr>
<th>Gene</th>
<th>Chr</th>
<th>Position</th>
<th>HGVS DNA Reference</th>
<th>HGVS Protein Reference</th>
<th>Variant Type</th>
<th>Pathogenicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>NUS1</td>
<td>6</td>
<td>6q22.31</td>
<td>NM_138459.5:g.117344163_119962769del</td>
<td>NP_612468.1</td>
<td>Deletion</td>
<td>Hap</td>
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</table>

**Table 4:** Description of Deleted OMIM Genes

<table>
<thead>
<tr>
<th>Genes</th>
<th>Description</th>
<th>Conditions</th>
<th>Pathogenicity</th>
</tr>
</thead>
</table>

7
<table>
<thead>
<tr>
<th>Gene</th>
<th>Description</th>
<th>Disorder/Condition</th>
<th>Pathogenicity</th>
</tr>
</thead>
<tbody>
<tr>
<td>VGLL2</td>
<td>Vestigial Like Family Member 2</td>
<td>Spindle Cell Rhabdomyosarcoma; Pleomorphic Rhabdomyosarcoma</td>
<td>Unknown</td>
</tr>
<tr>
<td>ROS1</td>
<td>ROS Proto-Oncogene 1, Receptor Tyrosine Kinase</td>
<td>Susceptibility to lung Cancer</td>
<td>Unknown</td>
</tr>
<tr>
<td>GOPC</td>
<td>Golgi Associated PDZ And Coiled-Coil Motif Containing</td>
<td>Spermatogenic Failure 9 and <em>Acute Laryngitis.</em></td>
<td>Unknown</td>
</tr>
<tr>
<td>NUS1</td>
<td>NUS1 dehydrodolichyl diphosphate synthase subunit</td>
<td>Congenital Disorder of Glycosylation, Type Ia, and autosomal dominant type 55 intellectual disability with seizures</td>
<td>Likely Pathogenic</td>
</tr>
<tr>
<td>CEP85L</td>
<td>Centrosomal Protein 85 Like</td>
<td>Lissencephaly 10 and Lissencephaly</td>
<td>Likely Pathogenic</td>
</tr>
<tr>
<td>PLN</td>
<td>Phospholamban</td>
<td>Cardiomyopathy, Dilated, 1P and Cardiomyopathy, Familial Hypertrophic, 18</td>
<td>Likely Pathogenic</td>
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<tr>
<td>MCM9</td>
<td>Minichromosome Maintenance 9 Homologous Recombination Repair Factor</td>
<td>Ovarian Dysgenesis 4 and Premature Ovarian Failure 1</td>
<td>Likely Pathogenic</td>
</tr>
<tr>
<td>ASF1A</td>
<td>Anti-Silencing Function 1A Histone Chaperone</td>
<td>Cataract 34, Multiple Types and Alpha-Thalassemia</td>
<td>Unknown</td>
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<tr>
<td>MAN1A1</td>
<td>Mannosidase Alpha Class 1A Member 1</td>
<td>Congenital Disorder Of Glycosylation, Type Iu</td>
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