Int J Mol Epidemiol Genet 2021;12(6):129-134
www.ijmeg.org /ISSN:1948-1756/IJMEG0139109

Case Report

Eye manifestations in the *NSUN2* intellectual disability syndrome

Graham Pingree[1], Amy Harper[2,3], Jordan Snajczuk[4], Natario L Couser[2,4,5]

[1]Virginia Commonwealth University School of Medicine, Richmond, VA, USA; [2]Department of Pediatrics, Virginia Commonwealth University School of Medicine, Children's Hospital of Richmond at VCU, Richmond, VA, USA; [3]Department of Neurology, Virginia Commonwealth University School of Medicine, Children's Hospital of Richmond at VCU, Richmond, VA, USA; [4]Department of Human and Molecular Genetics, VCU Health, Richmond, VA, USA; [5]Department of Ophthalmology, Virginia Commonwealth University School of Medicine, Richmond, VA, USA

Received September 13, 2021; Accepted November 10, 2021; Epub December 15, 2021; Published December 30, 2021

Abstract: The *NSUN2*-intellectual disability syndrome is a rare disorder of the cellular transcriptome that prevents proper t-RNA splicing. This disorder interrupts cellular function and leads to an accumulation of RNA fragments, producing a constellation of symptoms including dysmorphic facies, hypotonia, microcephaly, and short stature. Eye manifestations have been reported but not well characterized. Our study presents a new case involving a 4-year-old boy with novel *NSUN2* variants and clinical features consistent with the syndrome. In addition, through a systemic review, we discuss the 24 previously reported cases of the syndrome with an emphasis on the eye and ocular adnexa clinical features.

Keywords: NSUN2, NSUN2-related syndrome, NSUN2 intellectual disability syndrome, t-RNA modification, 5-cytosine methyltransferase

Introduction

The *NSUN2* intellectual disability syndrome (MIM #611091) is a rare disorder caused by mutations in post-transcriptional tRNA modification. Located at chromosome 5p15.31, the *NSUN2* gene encodes a 5-cytosine methyltransferase required for the proper splicing of an intron containing tRNA at C34 of tRNA-leu (CAA) and at C47 and C48 of tRNA-asp (GTC) [1, 2]. Blanco et al. suggests that failure of *NSUN2*-mediated tRNA methylation contributes to disease via accumulation of 5'tRNA-derived small RNA fragments [3]. These fragments trigger stress-induced RNA cleavage. Moreover, the accumulation of these fragments reduces translation rates, reduces cell size, contributes to infertility, may induce cyclic alopecia, and increases apoptosis of cortical, hippocampal, and striatal neurons [3].

First reported among consanguineous Iranian and Kurdish families, the *NSUN2* intellectual disability syndrome encompasses a large constellation of symptoms that include microcephaly, short stature, hypertelorism, intellectual disability (ID), short philtrum, full upper lip, hypotonia, delayed puberty, infertility, and additional dysmorphic features [4]. This initial description has since expanded to include juvenile cataracts, chronic nephritis, hearing impairment, seizures, cerebellar atrophy, and simplified gyral patterning of the frontal lobe [2, 5, 6]. Due to *NSUN2*'s ubiquitous role in the cell transcriptome, a wide range of phenotypic variability is expected. The *NSUN2* gene has additionally been implicated in oncogenic processes relating to cancers including head and neck squamous cell carcinoma and hepatocellular carcinoma [7, 8].

Specifically, *NSUN2* intellectual disability syndrome manifests in a wide array of ocular related symptoms. Hypertelorism, blepharophimosis, telecanthus, ptosis, long and down-slanting palpebral fissures, juvenile cataracts, strabismus, esotropia, and horizontal nystagmus have all previously been described. In this arti-

cle we describe the previously reported ocular manifestations of the *NSUN2*-related syndrome and present a new case involving a young male patient found to have two heterozygous variants in the *NSUN2* gene. In comparison to other reports, our patient demonstrates a milder case; however, hypotonia, global developmental delay, and intellectual disability were still present. Ocular symptoms included almond-shaped eyes, clogged tear ducts, epiblepharon, and strabismus. Although there are few cases reported to date, our report, to the best of our knowledge, is the first reported case of epiblepharon in an *NSUN2*-related syndrome.

Case report

We present a now four-year-old boy who was born by caesarean section at 40 weeks to non-consanguineous parents. The G1P1 37-year-old mother reported no pregnancy complications. At birth the patient was in 99th percentile for length; however, his head was measured in the 34th percentile, and his weight was in the 9th percentile. He passed a hearing screen at birth. Low tone was noted shortly after birth, and the patient had difficulty with feeding. Early feeding therapy improved his oral-motor dysfunction.

His mother first reported developmental delays when the child failed to roll over by six months and did not sit until nine months. At 12 months, it was discovered that the patient had several unique features including clinodactyly of the fifth digit and abnormal helices. During this period a chromosomal microarray demonstrated an atypical pattern in a palindromic-rich region of the patient's Y chromosome at Yq11.223 and Yq11.23, though a peripheral blood specimen showed no clear structural abnormality. An MRI of the brain at 15 months was unremarkable. The patient began to pull to stand at 14 months and first walked at 22 months. Strabismus and epiblepharon were noted at the same age.

Whole exome sequencing performed at age two revealed two heterozygous variants in the *NSUN2* gene: a pathogenic variant c.360-1G>C, IVS3-1G>C from his mother and a likely pathogenic variant c.787 G>T, p.D263Y from his father. The c.360-1G>C, IVS3-1g>C variant is a canonical splice site variant in a gene for which loss-of-function is a known mechanism of disease. The c.787G>T, p.Asp263Tyr variant was observed with a pathogenic variant on the opposite allele (in trans) in this patient. The in-silico analysis, which included protein predictors and evolutionary conservation, supported a deleterious effect. At the time of analysis, neither of the described variants had been previously published as pathogenic or benign.

By three years of age, the patient demonstrated a global developmental delay and was suspected to have intellectual disability. His motor skills, proprioception, and kinesthetic awareness were all at the level expected for a 24-month-old. His mother noted episodes of self-harm when he was mad or frustrated. After months of speech therapy, he showed signs of a mildly improved vocabulary; however, his cognitive scores remained greater than 1 SD below average. Currently at age four, the patient remains nonverbal and demonstrates chronic dysphagia, difficulty walking, and hearing problems. Spontaneous improvement in his epiblepharon was noted in both eyes.

Methods

We performed a systemic review of the literature to categorize the current body of knowledge surrounding eye manifestations in *NSUN2*-related disease. A PubMed/Medline search occurred in July of 2021, and our query included "*NSUN2*" and yielded 106 papers. The articles were mined for case reports of *NSUN2*-related syndromes as well as for additional references absent from our initial search.

Results

Our review of the literature yielded 8 articles describing 24 cases of *NSUN2*-related syndrome, which, together with our patient, brings the total number of report cases to 25 (**Table 1**). Fifteen of the reported 25 cases (60%) demonstrated ophthalmological disease, ranging widely: hypertelorism (n=7), strabismus (n=5), ptosis (n=4), blepharophimosis (n=4), telecanthus (n=3), long palpebral fissures (n=3), juvenile cataracts (n=2), epiblepharon (n=1), down slanting palpebral fissures (n=1), and horizontal nystagmus (n=1).

Abbasi-Moheb et al. reported three sets of consanguineous siblings with diverse *NSUN2* mutations. Of these three families, only one set of siblings with the g.6622224A>C variant was diagnosed with strabismus. The authors did not designate eye pathology in the other families [4].

Table 1. Summary of 25 patients with NSun2-related syndrome that have reported findings

Origin	Abbasi-Moheb et al. (2012)								Khan et al. (2012)		
	Iranian/Kurdish								Pakistani		
NSun2 gene pathogenic variant	c.679C>T (p.Gln227*)	c.679C>T (p.Gln227*)	c.679C>T (p.Gln227*)	c.1114C>T (p.Gln372*)	c.1114C>T (p.Gln372*)	c.1114C>T (p.Gln372*)	g.6622224A>C	g.6622224A>C	c.2035G>A (p.Gly679Arg)	c.2035G>A (p.Gly679Arg)	c.2035G>A (p.Gly679Arg)
Age	29	28	17	61	27	22	9	6	14	13	6
Gender	M	F	F	F	F	M	F	M	F	F	F
Eye findings	N.R.	N.R.	N.R.	N.R.	N.R.	N.R.	Strabismus	Strabismus	Strabismus	-	Esotropia and horizontal nystagmus
Intellectual Disability	+	+	+	+	+	+	+	++	+	+	+
Dysmorphic features	+	+	+	+	+	+	+	+	+	+	+
Microcephaly	+	-	-	+	+	+	+	+	+	+	N.R.
Short Stature	-	+	+	+	-	-	+	+	N.R.	N.R.	N.R.
Neurology	Muscular hypotonia	Muscular hypotonia	Muscular hypotonia	Muscular hypotonia	Muscular hypotonia	Muscular hypotonia	N.R.	N.R.	Muscular hypotonia, broad gait	Muscular hypotonia, broad gait	Muscular hypotonia
Delayed puberty	N.R.	N.R.	N.R.	N.R.	N.R.	N.R.	N.R.	N.R.	-	-	NA
Developmental Delay	N.R.	N.R.	N.R.	N.R.	N.R.	N.R.	N.R.	N.R.	N.R.	N.R.	N.R.
Feeding issues	N.R.	N.R.	N.R.	N.R.	N.R.	N.R.	N.R.	N.R.	N.R.	N.R.	N.R.

Origin	Martinez et al. (2012)			Fahiminiya et al. (2014)	Komara et al. (2015)	Mu et al. (2019)	Sun et al. (2020)			Kato et al. (2021)				Pingree et al. (2021)
	Lebanese			Qatari	Emirati	Emirati	Chinese			Japanese		Pakistani		United States
NSun2 gene pathogenic variant	g.6622-214C>G	g.6622-214C>G	g.66222-14C>G	c.915del (p.Gly306-Valfs*15)	c.1020-delA	c.1020-del (p.G341fs)	c.1004T>A (p.Leu335*)	c.1004T>A (p.Leu335*)	c.1004T>A (p.Leu335*)	c.546_547insCT, (p.Met183Leufs*13); c.1583del, (p.Pro528Hisfs*19)	c.546_547insCT, (p.Met183Leufs*13); c.1583del, (p.Pro528Hisfs*19)	c.1269-dup, (p.Val-424Cys-fs*14)	c.1269-dup, (p.Val-424Cys-fs*14)	c.360-1G>C (IVS3-1G>C); c.787G>T (p.D263Y)
Age	N.R.	N.R.	N.R.	6	16	21	20	4	4	16	11	16	12	3
Gender	M	F	F		M	M	F	F	F	F	M	F	M	M
Eye findings	Hypertelorism, blepharophimosis, telecanthus	Hypertelorism, blepharophimosis, telecanthus	Hypertelorism, blepharophimosis, telecanthus	Blepharophimosis, hypertelorism, bilateral ptosis	Down slanting palpebral fissures,	N.R.	Hypertelorism, ptosis, long palpebral fissures	Hypertelorism, ptosis, long palpebral fissures	Hypertelorism, ptosis, long palpebral fissures	Bilateral cataracts	Bilateral cataract	-	-	Bilateral epiblepharon, strabismus
Intellectual Disability	+	+	+	+	+	+	+	+	+	+	+	+	++	+
Dysmorphic features	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Microcephaly	+	+	+	N.R.	+	+	+	+	+	+	+	+	+	-
Short Stature	+	+	+	N.R.	+	+	+	+	+	+	+	+	+	-

Neurology	Axial hypotonia, epileptic seizures	Axial hypotonia	Axial hypotonia	Axial Hypotonia	Cerebellar atrophy, broad gait	Cerebellar hypoplasia	Muscular hypotonia	Muscular hypotonia	Muscular hypotonia	Hearing impairment	Hearing impairment	Hypomyelination, simplified gyral pattern of frontal lobe	Hypomyelination, simplified gyral pattern	Motor delay, difficulty swallowing, muscular hypotonia, hearing loss, shuddering spells
Delayed puberty	N.R.	N.R.	N.R.	N.R.	+	+	+	N.R.	N.R.	+	+	+	+	N.R.

* = translation stop codon; *M* = Male; *F* = Female; *N.R.* = Not Reported; + = Reported Feature.

Khan et al. described another set of three siblings with an identical c.2035G>A (p.Gly679-Arg) variant. The oldest sibling displayed unilateral strabismus, while the youngest sibling demonstrated intermittent esotropia and fine horizontal nystagmus. The middle child, however, was not diagnosed with ocular disease [9].

A study from Martinez et al. presented three patients from a single Lebanese family with the same g.6622214C>G mutation. Each of the three children were diagnosed with identical ocular features of hypertelorism, blepharophimosis, and telecanthus [2].

Fahiminiya et al. report the case of a 6-year-old boy born to first cousins of Qatari origin who developed multiple ocular diseases. The patient the c.915del (p.Gly306Valfs*15) variant had blepharophimosis, hypertelorism, and bilateral ptosis [10].

Komara et al. present a patient born of consanguineous parents who also developed eye disease. The patient who had a c.1020delA variant showed down-slanting palpebral fissures [11].

Kato et al. describes two sets of siblings, one of which was diagnosed with ophthalmological disease. One set of siblings of Japanese origin both possessed identical compound heterozygous frameshift variants: c.546_547insCT, (p.Met183Leufs*13) and c.1583del, (p.Pro528Hisfs*19). The siblings both developed juvenile cataracts. Of note, the brother and sister both displayed significant hearing impairment. The other set of siblings described possessed a c.1269dup, (p.Val424Cysfs*14) variant but did not display ocular disease or vision impairment [5].

An additional case did not report ocular disease [6].

Discussion

The exact mechanisms of the ocular pathologies relating to *NSUN2* variants are still widely unknown. The severity of the ophthalmological disease and overall clinical course is likely dependent upon the variants involved, although more cases will be needed to establish genotype-phenotype correlations. Nevertheless, it is suspected that due to the gene's foundational influence on cellular activity as a methyltransferase, many ocular pathways are likely affected through transcriptome variation, giving rise to the many phenotypic variations reported in this study. The most visual threatening clinical feature among those reported, bilateral juvenile cataracts, occurred in siblings with heterozygous frameshift mutations located at exons 6 and 14 (c.546_547insCT; c.1583del) that were considered to induce nonsense mediated decay (NMD) and decreased mRNA expression [5]. Adissu et al. reports that *NSUN2* knockout mice demonstrate cataract and abnormal lens morphology, while Chi et al. demonstrate that *NSUN2* is highly expressed both in the embryological brain and optical plate. Taken together, these studies suggest that *NSUN2*-related cataracts could result from abnormal optical plate development [12, 13].

We hypothesize that *NSUN2's* role in optical plate formation may explain the wide array of ocular disease morphologies. Further cytogenetic study of embryological morphology may elucidate the mechanism of ocular disease presented in this report. Moreover, additional testing may help explain the mechanism behind the improvements seen in our patient's ocular symptoms that are absent from other more severe cases described in the literature.

In summary, the eye manifestations of *NSUN2* intellectual disability syndrome present numerous pathologies with varying degrees of severity. In this report we describe novel pathogenic c.360-1G>C, IVS3-1G>C and a likely pathogenic c.787G>T, p.D263Y *NSUN2* gene variants. We also describe the resulting global developmental delay, feeding difficulties, hypotonia, strabismus, and epiblepharon. Although our patient demonstrated only mild ocular disease, numerous studies have recounted severe ocular diseases possible through *NSUN2* variants. We suggest that *NSUN2's* role in this wide variety of ocular pathologies is likely related to the gene's early embryological role in optical plate formation. Further study of optical plate morphology and cytogenetics is needed to elucidate the ocular disease process.

Disclosure of conflict of interest

We report that Dr. Couser is on the Patient-Centered Outcomes Research Institute (PCORI) Advisory Panel on Rare Disease and is involved in a clinical trial with Retrophin, Inc./Travere Therapeutics, Inc. Dr. Couser is also involved in a clinical trial with the National Cancer Institute/Children's Oncology Group and is a book

editor for Elsevier. Dr. Harper is involved in VCU contracted clinical trials with the CDC, NIH, Vertex Pharmaceuticals, Muscular Dystrophy Association, Fulcrum Therapeutics, Astellas Pharma, Reveragen, ML Biosolutions, Italafarmaco, and Novartis Gene Therapies. Additional research funding from Children's Hospital Research Grand and the VCU Center for Clinical and Translational Research. She is a chapter author for Elsevier. The authors note no other conflicts of interest and are solely responsible for the content and writing of this paper.

Address correspondence to: Dr. Natario L Couser, Department of Ophthalmology, Virginia Commonwealth University School of Medicine, Children's Hospital of Richmond at VCU, 1000 E Broad Street, Children's Pavilion, 6th FL, Suite K, Richmond, VA, USA. Tel: 804-828-9315; Fax: 804-628-9544; E-mail: natario.couser@vcuhealth.org

References

[1] Brzezicha B, Schmidt M, Makalowska I, Jarmolowski A, Pienkowska J and Szweykowska-Kulinska Z. Identification of human tRNA: m5C methyltransferase catalysing intron-dependent m5C formation in the first position of the anticodon of the pre-tRNA Leu (CAA). Nucleic Acids Res 2006; 34: 6034-6043.

[2] Martinez FJ, Lee JH, Lee JE, Blanco S, Nickerson E, Gabriel S, Frye M, Al-Gazali L and Gleeson JG. Whole exome sequencing identifies a splicing mutation in NSUN2 as a cause of a dubowitz-like syndrome. J Med Genet 2012; 49: 380-385.

[3] Blanco S, Dietmann S, Flores JV, Hussain S, Kutter C, Humphreys P, Lukk M, Lombard P, Treps L, Popis M, Kellner S, Holter SM, Garrett L, Wurst W, Becker L, Klopstock T, Fuchs H, Gailus-Durner V, Hrabe de Angelis M, Karadottir RT, Helm M, Ule J, Gleeson JG, Odom DT and Frye M. Aberrant methylation of tRNAs links cellular stress to neuro-developmental disorders. EMBO J 2014; 33: 2020-2039.

[4] Abbasi-Moheb L, Mertel S, Gonsior M, Nouri-Vahid L, Kahrizi K, Cirak S, Wieczorek D, Motazacker MM, Esmaeeli-Nieh S, Cremer K, Weissmann R, Tzschach A, Garshasbi M, Abedini SS, Najmabadi H, Ropers HH, Sigrist SJ and Kuss AW. Mutations in NSUN2 cause autosomal-recessive intellectual disability. Am J Hum Genet 2012; 90: 847-855.

[5] Kato K, Mizuno S, Morton J, Toyama M, Hara Y, Wasmer E, Lehmann A and Ogi T. Expanding the phenotype of biallelic loss-of-function variants in the NSUN2 gene: description of four individuals with juvenile cataract, chronic nephritis, or brain anomaly as novel complications. Am J Med Genet A 2021; 185: 282-285.

[6] Mu W, Schiess N, Orthmann-Murphy JL and El-Hattab AW. The utility of whole exome sequencing in diagnosing neurological disorders in adults from a highly consanguineous population. J Neurogenet 2019; 33: 21-26.

[7] Sun Z, Xue S, Zhang M, Xu H, Hu X, Chen S, Liu Y, Guo M and Cui H. Aberrant NSUN2-mediated m(5)C modification of H19 lncRNA is associated with poor differentiation of hepatocellular carcinoma. Oncogene 2020; 39: 6906-6919.

[8] Lu L, Zhu G, Zeng H, Xu Q and Holzmann K. High tRNA transferase NSUN2 gene expression is associated with poor prognosis in head and neck squamous carcinoma. Cancer Invest 2018; 36: 246-253.

[9] Khan MA, Rafiq MA, Noor A, Hussain S, Flores JV, Rupp V, Vincent AK, Malli R, Ali G, Khan FS, Ishak GE, Doherty D, Weksberg R, Ayub M, Windpassinger C, Ibrahim S, Frye M, Ansar M and Vincent JB. Mutation in NSUN2, which encodes an RNA methyltransferase, causes autosomal-recessive intellectual disability. Am J Hum Genet 2012; 90: 856-863.

[10] Fahiminiya S, Almuriekhi M, Nawaz Z, Staffa A, Lepage P, Ali R, Hashim L, Schwartzentruber J, Abu Khadija K, Zaineddin S, Gamal H, Majewski J and Ben-Omran T. Whole exome sequencing unravels disease-causing genes in consanguineous families in Qatar. Clin Genet 2014; 86: 134-141.

[11] Komara M, Al-Shamsi AM, Ben-Salem S, Ali BR and Al-Gazali L. A novel single-nucleotide deletion (c.1020delA) in NSUN2 causes intellectual disability in an emirati child. J Mol Neurosci 2015; 57: 393-399.

[12] Adissu HA, Estabel J, Sunter D, Tuck E, Hooks Y, Carragher DM, Clarke K, Karp NA; Sanger Mouse Genetics Project, Newbigging S, Jones N, Morikawa L, White JK and McKerlie C. Histopathology reveals correlative and unique phenotypes in a high-throughput mouse phenotyping screen. Dis Model Mech 2014; 7: 515-524.

[13] Chi L and Delgado-Olguin P. Expression of NOL1/NOP2/sun domain (Nsun) RNA methyltransferase family genes in early mouse embryogenesis. Gene Expr Patterns 2013; 13: 319-327.