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An expansion of the phenotype in individuals with SYNCRIP-Related Neurodevelopmental Disorder

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ABSTRACT

Disruption of genes within the *HNRNP* gene family has been observed in neurodevelopmental and neurodegenerative diseases. The HNRNP-Related Neurodevelopmental Disorders (HNRNP-RNDDs), while each unique, have been recently described with similar clinical and molecular features across variation in several genes. However, the phenotypic information on these patients is still lacking. In this case series we aim to describe the phenotypes that are associated with SYNCRIP-Related Neurodevelopmental Disorder (SYNCRIP-RNDD). We describe in depth ten novel individuals and one previously published individual with mostly *de novo* and predicted damaging variants in *SYNCRIP*, consistent with a diagnosis of SYNCRIP-RNDD. We also describe previously published patients, many of which are from large cohort studies, as well as individuals from patient databases. Here, we expand the phenotype of SYNCRIP-RNDD beyond a generic neurodevelopmental disorder to a variable syndrome consisting of mild to borderline developmental delay/intellectual disability, speech and language delay, behavioral differences such as autism spectrum disorder, structural brain anomalies, hypotonia, and seizures. Inconsistent dysmorphic features were also observed, with the few recurrent findings including long

Abbreviations: HNRNP, Heterogeneous nuclear ribonucleoprotein.

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eyelashes, mildly deep-set eyes, prominent ears, and thin or thick lips. This study increases our understanding of SYNCRIP-RNDD, as well as HNRNP-RNDDs broadly.

1. Introduction

Many of the 7,000+ rare diseases are monogenic neurodevelopmental disorders (NDDs). Over 1,000 genes encode RNA binding proteins (RBPs), many of which have been associated with NDDs, most notably FMRP with fragile X syndrome (MIM: 300624). The heterogeneous nuclear ribonucleoprotein (*HNRNP*) gene family has been associated with neurodegenerative disorders, cancer, and more recently with NDDs [1–29]. hnRNPs function as RBPs playing roles in mRNA processing such as RNA maturation, stabilization, and translation [14,30]. The NDDs resulting from variants in *HNRNP* genes, the HNRNP-Related Neurodevelopmental Disorders (HNRNP-RNDDs), have been shown to have clinical overlap, with patients having developmental delay/intellectual disability (DD/ID), epilepsy, behavioral differences such as autism spectrum disorder (ASD), and congenital anomalies [1–6,15,19]. Several of the HNRNP-RNDDs were identified in the clinic, while some were identified through statistical analysis of variation among large NDD cohorts. To date, the HNRNP-RNDDs include HNRNPC-RNDD (MIM: 620688), RBMX/HNRNPG-RNDDs (also known as Gustavson Syndrome [MIM 309555] and Shashi type ID [MIM 300238]), HNRNPH1-RNDD (MIM: 601035), HNRNPH2-RNDD (also known as Bain-type ID, MIM: 300986), HNRNPK-RNDD (also referred to as Au-Kline or Okamoto syndrome, MIM: 616580), HNRNPR-RNDD (MIM: 620073), HNRNPU-RNDD (MIM: 617391), and SYNCRIP/HNRNPQ-RNDD [1–6,8,10–12,15,19,21,22,25,27,29,31–43]. Several other *HNRNP* genes (including *HNRNPD, HNRNPL, HNRNPUL1,* and *HNRNPUL2*) have been proposed as NDD candidate genes as well and are actively being characterized.

The *HNRNP* gene family consists of over 30 members, designated A through U or as hnRNP-like proteins. SYNCRIP, also known as hnRNPQ, is an RBP involved in neuronal RNA transport, microRNA processing, and gene expression. Highlighting its role in neurological disease, *SYNCRIP*, or synaptotagmin-binding cytoplasmic interacting protein, was first identified in the brain [44]. Studies in *Drosophila* have implicated alteration of the fly homolog of *SYNCRIP*, *Syp*, in behavioral traits and in maintaining a balance between presynaptic vesicle release and postsynaptic translation [45]. In mammalian neuronal cells, the cytoplasmic RNA interactome of SYNCRIP consists of target genes involved in neuronal differentiation [46]. While ubiquitously expressed, *SYNCRIP* has been shown to be highly expressed in the brain, particularly during development [15].

SYNCRIP has been identified as a gene with statistically significantly enriched rare and predicted pathogenic *de novo* variation among large NDD cohorts [15,47,48]. Additionally, SYNCRIP likely plays roles in other NDDs. Of the many genes lost through proximal 6q deletions, *SYNCRIP* has been suggested to play a significant role in the neurodevelopmental phenotypes associated with 6q deletion syndrome (MIM: 613544) [49]. It has also been shown to be critical in internal ribosome entry site-mediated translation of *fmr1* in murine models of fragile X syndrome [50]. Finally, variation in *SYNCRIP* itself has been shown to cause a neurodevelopmental syndrome consisting of DD/ID, ASD, speech delays and difficulties, and hypotonia [15,25]. We present a case series of ten novel patients and one previous published individual with additional information with SYNCRIP-RNDD in addition to review of the literature and patient databases to further delineate these findings and assess genotype-phenotype relationships.

2. Methods

2.1. Novel patients

All patients are novel except Patient 9 who has been previously published [15]. All research carried out on these individuals were in accordance with the ethical standards of the responsible committee on human research (institutional and national), and proper informed consent was obtained phenotypic workup. This study is approved by Columbia University's and University of Calgary's Institutional Review Boards (IRBs).

Several patients were identified through GeneMatcher [51]. When families from large cohort studies or through GeneMatcher could not be contacted, deidentified clinical information was used. Probands were excluded if they reportedly had a variant observed in gnomAD v4.1.0 more than once and/or a missense variant with a CADD (v1.7, GRCh38) score < 20 [52].

2.2. Literature and database review

Published cases with variants in *SYNCRIP* were identified from large NDD cohort studies and case reports by searching PubMed with all known gene aliases (*SYNCRIP* and *HNRNPQ*). Cohorts include all of those from Gillentine et al. [15] (the Simons Simplex Collection (SSC), MSSNG, the Epi4K Consortium, Autism Sequencing Consortium (ASC), Epilepsy Genetics Initiative, Deciphering Developmental Disorders (DDD4K and DDD13K), Baylor Genetics Laboratory (BGL), and the SPARK Consortium) and any known new patients from updates of those cohorts. Neurodegenerative cohorts were not included. Duplicates were removed based on sample ID, sharing of variants, and disclosure of duplicates in publications. ClinVar, DECIPHER, and LOVD were also queried. Variants included in HGMD Professional 2023.4 were reviewed as well.

2.3. Variant interpretation

All variants were queried by multiple *in silico* tools using MobiDetails (https://mobidetails.iurc.montp.inserm.fr/)[53], including CADD[52], SpliceAI and SpliceAI Visual [54], MetaDome, REVEL, PolyPhen, SIFT, AlphaMissense, and MPA. Variants were also queried with MutationTaster2021 and SpliceVault [55]. CADD scores were estimated for frameshift insertions by using the maximum of all SNV scores for both flanking bases, for frameshift deletions using the maximum of all SNV scores for all spanning bases, for in-frame insertions using the average of all SNV scores for both flanking bases, and for in-frame deletions using the average of all SNV scores for all spanning bases. For frameshift variants, likelihood of nonsense mediated decay (NMD) was determined by NMDEscPredictor [56].

2.4. Protein isolation and western blot

SYNCRIP-RNDD patient skin biopsies were procured at Columbia University Irving Medical Center under IRB oversight. Dermal fibroblasts were derived from skin biopsies by the Columbia University Stem Cell Core as previously described [57]. One male, Patient 3, age 6 years at the time of donation with the NM_006372.4:c.415_418dup, p.Gly140Aspfs*25 variant, and one female, Patient 9, age 13 years at the time of donation with the NM_006372.4:c.1299 T>G, p.Tyr433* variant, were used. In addition, a primary dermal fibroblast line from an African American female, age 31 years, was used as a reference control (ATCC®- Number - PCS-201–012™).

Fibroblasts were thawed, maintained, and grown in six well tissue culture treated plates (Corning Costar® #3506). Cells were passaged and grown (n = 3) for 6–8 days before protein isolation. Cells were lysed in IP Lysis Buffer (ThermoFisher #87787) containing protease and phosphatase inhibitors (ThermoFisher #78442). Lysis occurred for 20 minutes on ice with intermittent vortexing. Cell debris was removed by centrifuging the lysate at 16,000 g for 10 minutes and the total protein content of the supernatant was quantified using Pierce BCA kit. Lysates were mixed with 4x Laemmli buffer, reducing (4X) (ThermoFisher #J60015.AD), and boiled at 95 ℃ for 5 minutes and ~19–23 μg total protein was loaded in a 4–20 % Tris-glycine gel (ThermoFisher, XP04205BOX). The gels were transferred onto 0.2 μm nitrocellulose membranes, and blocked in 5 % non-fat dry milk. Incubation with hnRNP Q/R antibody (Cell Signaling Technology hnRNP Q/R (D18B2) Rabbit mAB (#8588), 1:1000) was done overnight at 4°C in 5 % BSA in TBST. The following day, the membranes were washed and blotted with rabbit secondary antibody (Cell Signaling Technology, #7074, 1:5000) in milk for 1 h at room temperature. Western blot membranes were imaged on a LI-COR Odyssey Fc system, after incubation with ECL (ThermoFisher, SuperSignal West Femto Maximum Sensitivity Substrate #34094), and multipel band intensities together were quantified in the ImageStudio Lite Software (v5.2). Incubation with β-Actin (13E5) Rabbit mAb (HRP Conjugate) #5125 Beta-Actin (Cell Signaling Technology, #5125, 1:1000) for 1 h at RT was used as loading control on the same membrane.

3. Results

3.1. Case presentations

Patient 1 is a 15-year-old girl with growth delay including body weight and stature two standard deviations below average and mild DD/ID. Prenatal information was unavailable. She walked at 18 months and her first words were at 2-years-old. Her parents reported that she did poorly in school, had periods of inattention with blank stares, low energy, is slow moving, and has nocturnal enuresis. Exome sequencing revealed a *de novo* nonsense variant in *SYNCRIP* predicted to result in nonsense mediated decay (NMD) and haploinsufficiency (NM_006372.4:c.247_248del, p.(Ser83*)) (Tables 1 and 2).

Patient 2 is a 12-year-old female with a history of intrauterine growth restriction and failure to thrive, borderline IQ with DD, attention deficit hyperactivity disorder (ADHD), and hypotonia. She spent four days after birth in the neonatal isolation unit (NICU) and received phototherapy for two days. Her full-scale IQ is 78 with a verbal comprehension index of 86, fluid reasoning score of 82, visual spatial score of 97, and working memory score of 62. She attends mainstream school with additional resources. She walked at 13–15 months and had her first words at 16–18 months with sentences at 2–3 years. Upon physical examination she was noted to have mild bilateral 2–3 toe syndactyly and mottling of toes, myopia, striking green eyes, low-set and posteriorly rotated ears, and a thin upper lip. Exome sequencing revealed a *de novo* splice variant in *SYNCRIP* predicted to result in loss or gain of a splice acceptor site (NM_006372.4:c.268–1 G>C). Per SpliceVault, this most likely results in an out-of-frame protein product due to use of a cryptic splice site (+8) or due to skipping of exons 3 and 4, which would be expected to be subjected to nonsense mediated decay (NMD) and thus, result in haploinsufficiency [55]. There is also the possibility that this variant results in an in-frame protein but skips exon 4, removing part of the acidic domain of the protein, which may result in decreased or complete loss-of-function.

Patient 3 is a 7-year-old male with hypotonia, speech delay, and autistic features (Fig. 1). He was born full term with a birthweight of 3.4 kg. His birth history is complicated by a 4-hour NICU stay for oxygen desaturation. He also had a pyloromyotomy at four weeks of life. Pregnancy was complicated with cholestasis of pregnancy. Developmental milestones were achieved on time; he smiled at 6–8 weeks, walked at 13 months, crawled at 6 months, and had single word speech at 12 months. He currently speaks in full sentences, which improved after treatment for absence seizures. Absence seizures were identified at seven years of age. His vision, hearing, and appetite are normal. He also hassleep disturbances. His brain MRI findings were significant for cortical dysplasia. Hypermobility was noted. Enuresis was noted and there was concern for neurogenic bladder. He has a history of recurrent infections. His family history is significant for a psychotic episode in his paternal uncle, his father and sister have ADHD, and there are extended family members with ASD. Physical examination was significant for down slanting palpebral fissures, long eyelashes, somewhat deep-set eyes, a flat malar region, a relatively small mouth with a full lower lip, and an open-mouthed appearance. Exome sequencing revealed a frameshift variant in *SYNCRIP* that is assumed to be *de novo*, although three reads containing the variant were identified in the paternal sample, and it is expected to result in NMD and haploinsufficiency (NM_006372.4:c.415_418dup, p.(Gly140Aspfs*25)).

Patient 4 is a 9-year-old male with global developmental delay, regression, and epilepsy. He was born full term through cesarean section with birthweight of 3.4 kg. Pregnancy was complicated with mother being exposed to an abusive partner and mother's systemic lupus erythematosus disease complexities. He started crawling at one year of age and walking at the age of two. He had plagiocephaly and macrocephaly and MRI done in this context revealed hydrocephalus. He had onset of tonic, atonic, and absence seizures at the age of 8 and is currently taking perampanel, but still has several seizures daily. Previously, he has been on several antiepileptic drugs. At the age of 5, he had a cystectomy in the setting of ganglion cysts. The disease initially presented with a cyst in the left foot which later progressed in size and multiple cysts appeared around the body. Prior to cystectomy surgery he was walking, but after surgery he had motor regression and lost complete ambulation by the age of 6. There was also social and cognitive decline along with language regression. MRI was done at this time, but it was reported to be normal. By parental report, doctors have said that he has a juvenile ALS-like phenotype. Other medical history consists of hypospadias at birth repaired at one year of age, eosinophilic esophagitis, aspiration, sensitive and thin skin, and tremor in his hands. Current medications include carbidopa/levodopa, gabapentin, pregabalin, diazepam, perampanel, clonazepam as needed for seizure rescue, ondansetron, glycopyrrolate, hydromorphone, clonidine, trazodone, and duloxetine. He has four maternal half siblings, two of which have ASD. He lives with mother and siblings and is home schooled along with his siblings. Exome sequencing revealed a *de novo* in-frame amino acid deletion in the first RNA recognition motif (RRM1) of SYNCRIP (NM_006372.4:c.641_643del, p.(Glu214del)). Notably, this variant is present in gnomAD v4.1.0 (1 of 1,609,942 alleles), as is another missense change at the same position (p.(Glu214Ala), 7 of 1,610,068 alleles). This may suggest that there is incomplete penetrance or other genetic or environmental factors influencing this individual's clinical features.

Patient 5 is a 10-year-old male with speech delay and hyperphagia. He was born 10 days overdue through cesarean section due to a nuchal cord. He had a birthweight of 2.7 kg and was discharged home without requiring any neonatal intensive care unit stay. Pregnancy was complicated with maternal cannabis use but no other concerns and prenatal scans were normal. Developmental milestones were achieved on time; he crawled at 10–11 months and walked close to his first birthday. Speech was delayed and he currently receives speech therapy. He has little sense of danger. His medical history includes obesity, umbilical hernia, tantrum behavior, food seeking behavior, somniloquy, and corrective glasses for astigmatism and hypermetropia. Significant physical examination findings are up slanting palpebral fissures, protruding ears, and upturned nose. He lives with his great aunt under special guardianship and attends 4th grade with 1-on-1 teaching. Clinical singleton genome sequencing revealed a deletion predicted to result in loss of exons 7 and 8 of *SYNCRIP* and result in an in-frame deletion (NM_006372.5:c.667–5696_1009–1323del, p.(Tyr223_Leu340del)),

Table 1
Current study patient variants.

Column1	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10	Patient 11
Variant (NM_006372.5)	c.247_248del, (Ser83*)	c.268–1 G>C	c.415_418dup, p.(Gly140Aspfs*25)	c.641_643del, p.(Glu214del)	c.667–5696_1009–1323del, p.(Tyr223_Leu340del)	c.858_859del, p.(Gly287Leufs*5)	c.945dup, p.(Asn316Glufs*6)	c.1235 A>G, p.(Arg412Lys)	c.1299 T>G, p.(Tyr433*)	c.1567 T>C, p.(Tyr523His)	c.1636 A>T, p.(Arg546*)
Genomic Coordinates (hg38)	chr6:85640465_85640466del	chr6:85640329 C>G	chr6:85637314_85637317dup	chr6:85636993_85636995del	chr6:85620741_85629809del	chr6:85622633_85622634del	chr6:85622549dup	chr6:85618863 T>C	chr6:85615329 A>C	chr6:85615061 A>G	chr6:85614992 T>A
Exon	3	Intron 3	5	5	7 and 8	8		10	11	11	11
CADD	28.8	35	40	41	-	-	-	24.4	38	24.1	39
SpliceAI	-	0.93/0.02	-	-	-	-	-	-	-		
Splice Impact	-	acceptor loss or acceptor gain, out of frame protein product	-	-	-	-	-	-	-	-	-
MetaTolerance Score	-	-	-	0.62	-	-	-	0.09	0.25	0.64	0.73
MetaTolerance Impact	-	-	-	slightly intolerant	-	-	-	highly intolerant	intolerant	slightly intolerant	neutral
REVEL	-	-	-	-	-	-	-	0.4	-	0.161	-
PolyPhen Hum Div	-	-	-	-	-	-	-	0.65	-	0.22	-
SIFT	-	-	-	-	-	-	-	0.19	-	0.1	-
MutationTaster 2021	Deleterious	-	Deleterious	Deleterious	-	Deleterious	Deleterious	Benign	Deleterious	Benign	Deleterious
Alpha Missense Score	-	-	-	-	-	-	-	0.236	-	0.357	-
MPA	10	10	10	-	-	-	-	4.44	10	2.22	10
NMD	Yes	-	Yes	No	No	Yes	Yes	No	Possibly	No	Possibly
Domain	-	-	-	RRM1	RRM1-RRM3	-	-	-	-	Disordered, region that interacts with SMN	Disordered
Population Databases	-	-	-	in gnomAD 4.1.0 1x (1/1,609,942 alleles)	-	in gnomAD v4.1.0 1x (1/1613,132 alleles)		-	-	in gnomAD v4.1.0 1x (1/1613,968 alleles)	-

Table 2
Current study patient phenotypes.

Column1	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10	Patient 11
CRID	N/A	N/A	C3BSUZ82	JJ23AH5P	N/A	686XUPUD	N/A	N/A	HY6QV5PQ	N/A	N/A
Variant (NM_006372.5)	c.247_248del, p.(Ser83*)	c.268–1 G>C	c.415_418dup, p.(Gly140Aspfs*25)	c.641_643del, p.(Glu214del)	c.667–5696_1009–1323del, p.(Tyr223_Leu340del)	c.858_859del, p.(Gly287Leufs*5)	945dup, p.(Asn316Glufs*6)	c.1235 A>G, p.(Arg412Lys)	c.1299 T>G, p.(Tyr433*)	c.1567 T>C, p.(Tyr523His)	c.1636 A>T, p.(Arg546*)
Inheritance	de novo	de novo	de novo, possibly mosiac in father	de novo	unknown	de novo	de novo	de novo	de novo	not maternal	unknown
Sex/Age (years)	F/15	F/12	M/7	M/9	M/10	M/4	M/3	M/4	F/13	F/12	M/9
Growth	growth delay	growth delay	normal	normal	normal	normal	height and weight >94th % ile	normal	growth delay	height and weight >99 %ile	height and weight <2 % ile
DD/ID	mild	borderline	DD	+	-	DD	GDD	DD	+	borderline IQ (~80)	moderate
Motor Delay	+	+	+	+	-		+	+	+	-	N/A
Age of first steps	18 months	13–15 months	13 months	2 years	1 year		close to 24 months	18 months	19 months	N/A	N/A
Motor Issues	slow moving	-	-	nonambulatory	-		-	-	wide gait, ataxia, poor balance	-	-
Speech Delay	+	+	+	-	+	+	+	-	+	+	+
Age of first words	2 years	16–18 months	12 months	N/A	N/A		spoke in single words at 3 years, diagnosed with moderate speech apraxia. Can follow single step commands	post regression primarily nonverbal with few inconsistent words	primarily nonverbal, only uses a few words, also has dysarthria	N/A	nonverbal
Regression	-	-	-	social, cognitive, and language regression	-	-	-	in language	-	in language skills at 3 years of age	-
Hypotonia	-	+	+	-	-		-	-	+	-	+
ASD	-	-	autistic features	-	-	+	autistic features	+	+	+	+
ADHD/ Attention Issues	+	+	-	-	-		+	+	-	-	-
Sleep Disturbances	-	-	+	somniloquy	-		-	+	+	+	+
Abnormal Tantrums	-	-	-	-	+		-	-	-	-	-
Structural Brain Anomalies	N/A	N/A	+	+	N/A		extensive Chiari Malformation	N/A	Chiari Malformation	N/A	N/A
Seizures	-	-	+	+	-		one febrile seizure	possibly	-	-	-
Vision Impairment	-	-	-	-	hypermetropia, astigmatism	astigmatism		hyperopia, astigmatism		-	-
Hearing Impairment	-	-	-	-	-	-	-	moderate unilateral conductive hearing loss	mild hearing loss	-	-
Dysmorphic Features	-	2–3 toe syndactyly and mottling	hypotonic open-mouth apperance,	macrocephaly, plagiocephaly, ganglion cysts	upslanting palpebral fissures, protruding ears, and upturned nose		square facial shape with prominent	epicanthus	prominent teeth, lip and tongue tie	-	oval face

(*continued on next page*)

Table 2 (*continued*)

Column1	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Patient 7	Patient 8	Patient 9	Patient 10	Patient 11
		of toes, myopia, striking green eyes, low-set and posteriorly rotated ears, and a thin upper lip	downslanting palpebral fissures				forehead, prominent jaw, deep set eyes				
Feeding Issues	-	-	-	-	-		-	+	poor due to hypotonia, previous G-tube, cyclical satiety problems possibly	-	G-tube, history of feeding difficulties
Neurogenic Bladder	-	-	possibly	-	-		-			-	-
Other	nocturnal enuresis		drooling, pyloric stenosis at 4 weeks	hypospadias, eosinophilic esophagitis, aspiration, sensitive and thin skin, tremor in his hands	no sense of danger, obesity, food seeking behavior			retractile testes, oppositional and anxious behavior	bradycardia and pulmonary stenosis at birth, concern for neurogenic bladder, laryngomalacia	psychosis	hypertonia

Fig. 1. Photos of Patient 3 at ages A) 1 year and B) 6 years. Notable features include his hypotonic open-mouth appearance at a younger age, and down slanting palpebral fissures.

although this was not confirmed by an orthogonal method [55]. This variant is predicted to result in loss of the entirety of the second RNA recognition motif (RRM2) and inheritance was not determined.

Patient 6 is a 4-year-old male with DD, ASD, and speech delay. He is a dizygotic twin; his twin sister is unaffected. Fetal ultrasound and postnatal MRI showed ventriculomegaly/enlarged ventricles, hypoplastic pituitary gland and pituitary stalk, absent septum pellucidum, a spine syrinx, and a small caliber corpus callosum. After birth, he was noted to have feeding difficulties and needed thickened formula. He was hypotonic. His motor milestones were delayed, with sitting at 9–10 months, crawling at 12 months, and walking at 22 months. His fine motor delay is also significant, with using a pincer grasp at 1 year but still using a raking grasp. He babbled at six months, had 1–2 word phrases at 2–2.5 years, and 5–10 words at 3.5 years of age, although these were primarily scripted. He is in the 97th percentile for height and head circumference, but this is consistent with his parents' features. Hypermobility is notable. He has persistent startle that has been responsive to CBD oil. Routine EEG showed epileptiform discharges and he had myoclonic blinking at 18 months, but to date, he has not had any noticeable seizure activity. While not significantly dysmorphic, he has persistent fetal pads, soft skin, long eyelashes, somewhat deep-set eyes, prominent ears superiorly, and a small mouth with a full lower lip and widely spaced teeth. He also has bilaterial hyperopia. He previously had mild dilation of one kidney, but this has resolved. When ill he exhibits laryngomalacia and stridor. He has been noted to have heat intolerance. Exome sequencing revealed a *de novo* loss-of-function variant in *SYNCRIP* (NM_006372.4:c.858_859del, p.(Gly287Leufs*5))

Patient 7 is a 3-year-old male with global developmental delay, mild cognitive disability, mild speech delay, attention-deficit, and autistic features. At 3 years he had 50–100 words but only spoke in single words and is diagnosed with moderate speech apraxia. His receptive language was also delayed, with only being able to follow single step commands. He is tall and overweight, with both height and weight being above the 94th percentile. His first steps were at close to two years of age. It is anticipated he will require additional resources once he starts school and currently receives speech and occupational therapy. He has had a single febrile seizure and has an extensive Chiari malformation identified by brain MRI. He has a square facial shape with a prominent forehead, prominent jaw, and deep-set eyes. Panel testing identified a *de novo* frameshift variant in *SYNCRIP* (NM_006372.4:c.945dup, p.(Asn316Glufs*6)).

Patient 8 is a 4-year-old male with DD, ASD, attention-deficit, oppositional and anxious behaviors, and sleep disturbances. Pregnancy was complicated by pre-eclampsia, nausea, and vomiting. He walked at 18 months and had normal language development until 18 months. This was followed by notable regression in language, and he is currently nonverbal with the exception of a few words on an inconsistent basis. Staring spells concerning for seizures were noted. He has bilateral hyperopia, astigmatism, and moderate unilateral conductive hearing loss. Upon physical examination he was noted to have epicanthus, retractile testes. Feeding difficulties were noted. He recurrently has otitis media. Exome sequencing revealed a *de novo* missense variant in *SYNCRIP* (NM_006372.4:c.1235 A>G, p.(Arg412Lys)).

Patient 9 was previously published with limited phenotypic information [15]. She is a 13-year-old female with ASD, ID/DD, hypotonia, and mild hearing loss. Pregnancy was uncomplicated and prenatal scans were normal. She was born full term at 40 weeks with a birthweight of 3.2 kg. Neonatal period was complicated by a NICU stay followed by a special care baby unit stay for bradycardia and a valvuloplasty intervention for pulmonary stenosis. Developmental milestones were delayed; she sat at 10 months and walked at 19 months. Her first spoken words were at 19 months, and she currently only speaks a few words. She has undergone a brain MRI which shows a Chiari malformation and a renal ultrasound which was normal. Her medical history includes poor feeding secondary to hypotonia resulting in G-tube feeding, cyclical satiety problems, two Chiari decompressions, surgical intervention for velopharyngeal insufficiency, neurogenic bladder, dysarthria, laryngomalacia, and tonsillectomy/adenoidectomy. To date she has not had any seizure activity. She has a history of poor balance, ataxia, and a wide gait. Medications currently used by the patient are gabapentin and oxybutynin for neurogenic bladder and gabapentin for sleep concerns. She is currently under the care of the following subspecialties: cardiology, neurology, neurosurgery, orthopedics, urology and ENT. Her family history is noncontributory. Physical examination showed prominent teeth and lip and tongue tie. She underwent microarray and karyotype testing for 22q11 deletion syndrome, both were negative. Exome sequencing revealed a *de novo* nonsense *SYNCRIP* variant (NM_006372.4:c.1299 T>G, p.(Tyr433*)).

Patient 10 is a 12-year-old female with ASD, psychosis, and overgrowth in both weight and height. Pregnancy was complicated by insulin treated gestational diabetes and hypertension in the mother. She was born at 39 weeks with a birth weight of 3 kg. Development was grossly normal but regression in language skills were noted at 3 years of age. She received speech therapy which improved her functional speaking skills. Her diagnosis of ASD occurred at eight years, and other than sleeping difficulties no other behavioral differences have been observed. She has a borderline IQ around 80 and is in special education as of age 12 years due to psychosis. Autism/ID gene panel testing identified a missense variant in *SYNCRIP* (NM_006372.4:c.1567 T>C, p.(Tyr523His)) that was determined to be not maternal. A paternal sample was not available. Her family history includes a sister with ASD and two grandmothers with episodes of psychosis, although none of these family members were assessed for the variant.

Patient 11 is a 9-year-old male with moderate ID, stature and weight below the second percentile, hypotonia and hypertonia, ASD, and sleep issues including obstructive sleep apnea. He has a history of feeding difficulties and constipation and uses a G-tube for feeding. He is nonverbal. He also has mild aortic root dilation and a large aortic valve annulus. The only dysmorphic feature noted is an oval face. He was adopted and clinical information prior to five years of age is unavailable, although his parents were reportedly consanguineous. Exome sequencing revealed a nonsense variant in *SYNCRIP* (NM_006372.4:c.1636 A>T, p.(Arg546*)).

3.2. Literature review, patient databases, and large population studies

Thirty-three cases were identified in the literature (Supplementary Table 1). Fifteen frameshift variants, ten missense variants, three nonsense variants, two splice variants, two indels, and two large deletions were identified [15,25,47,58–64].

Patient databases (ClinVar, DECIPHER, and LOVD) were also reviewed, identifying 23 individuals that had not been previously published (Supplementary Table 2). Variants predicted to cause loss of function and/or with CADD scores greater than 20 were included. All of these cases had unknown inheritance except for two, one of which was *de novo* and one was paternally inherited. Limited clinical information was available. Seven cases only were noted to have "inborn genetic disease". Case SCV001480245.1, carrying a missense variant, reportedly has short stature, DD/ID, seizures, and cerebral palsy. Notably, many HNRNP-RNDD patients are initially misdiagnosed with cerebral palsy. Case SCV004027820.1, carrying a frameshift variant, reportedly has no growth abnormalities, mild DD/ID, motor delay with a dystonic and movement disorder, dysarthria, abnormal behavior, and cleft palate. Case SCV002318760.1, carrying a nonsense variant, reportedly has DD/ID, hypertensive disorder, stage 5 chronic kidney disease, and was noted to have SYNCRIP-related neurodevelopmental disorder. Case SCV001432854.1, with a paternally inherited missense variant, reportedly has DD/ID. Both SCV004112895.1 and SCV004106336.1 have no clinical information but are listed in ClinVar with "SYNCRIP-related condition". Similarly, SCV004035858.1 was only listed as "affected." Case 407065 from DECIPHER carries a *de novo* frameshift variant and is a four-year-old male with normal growth, DD/ID, motor delay, speech delay, autistic features, hypotonia, seizures, microcephaly, shallow orbits, and hypopigmentation of his skin and hair. Finally, the case from LOVD has a nonsense variant but no clinical information available.

GnomAD v4.1.0, the *All of US* database, and the UK BioBank were all reviewed, identifying 29 predicted haploinsufficient or protein truncating variants affecting the *SYNCRIP* MANE transcript (NM_006372.4) (Supplementary Table 3). Two variants were included in the gnomAD v3.1.2 non-neuro samples, although gnomAD v4.1.0 does not include a non-neuro cohort at this time, so some of the individuals identified may have neurodevelopmental phenotypes. Most phenotypic information was not available but both *All of Us* and UK Biobank are known to include affected individuals, so it is assumed some of these individuals are affected. The *All of Us* samples (n = 4) had some data available through controlled access of the database. Among these individuals, anxiety and depression were reported in two individuals each, but they had no other neurological abnormalities. The presence of variants in these cohorts suggests there are more mildly affected individuals or that variable penetrance is possible, and anecdotally there have been cases inherited from an independently living affected parent. Four individuals in large population studies have variants that are predicted to be subjected to NMD. Eight individuals have variants that are predicted to escape NMD and result in a truncated protein product. Six individuals have protein products that are either elongated or degraded by non-stop RNA decay. Twelve variants cannot have their NMD status reliably predicted.

3.3. Genotypic spectrum of SYNCRIP-related neurodevelopmental disorder

The majority of variants in *SYNCRIP* among individuals in the literature and patient databases with known inheritance occurred *de novo* (84.2 %, n = 32/39). One each were maternal and paternally inherited, although no phenotypic information was available for the parents. Three had inheritance from one parent excluded. One case is assumed *de novo*, although three reads were identified carrying the variant in the patient's father (Patient 3).

Including all variants from the current study, the literature, and patient databases, 29 are missense variants, 21 are frameshift variants, eight are nonsense variants, three are splicing variants (two predicted to result in in-frame protein products, one predicted to undergo NMD), four are in-frame deletions/insertions, and two are multi-exon deletions

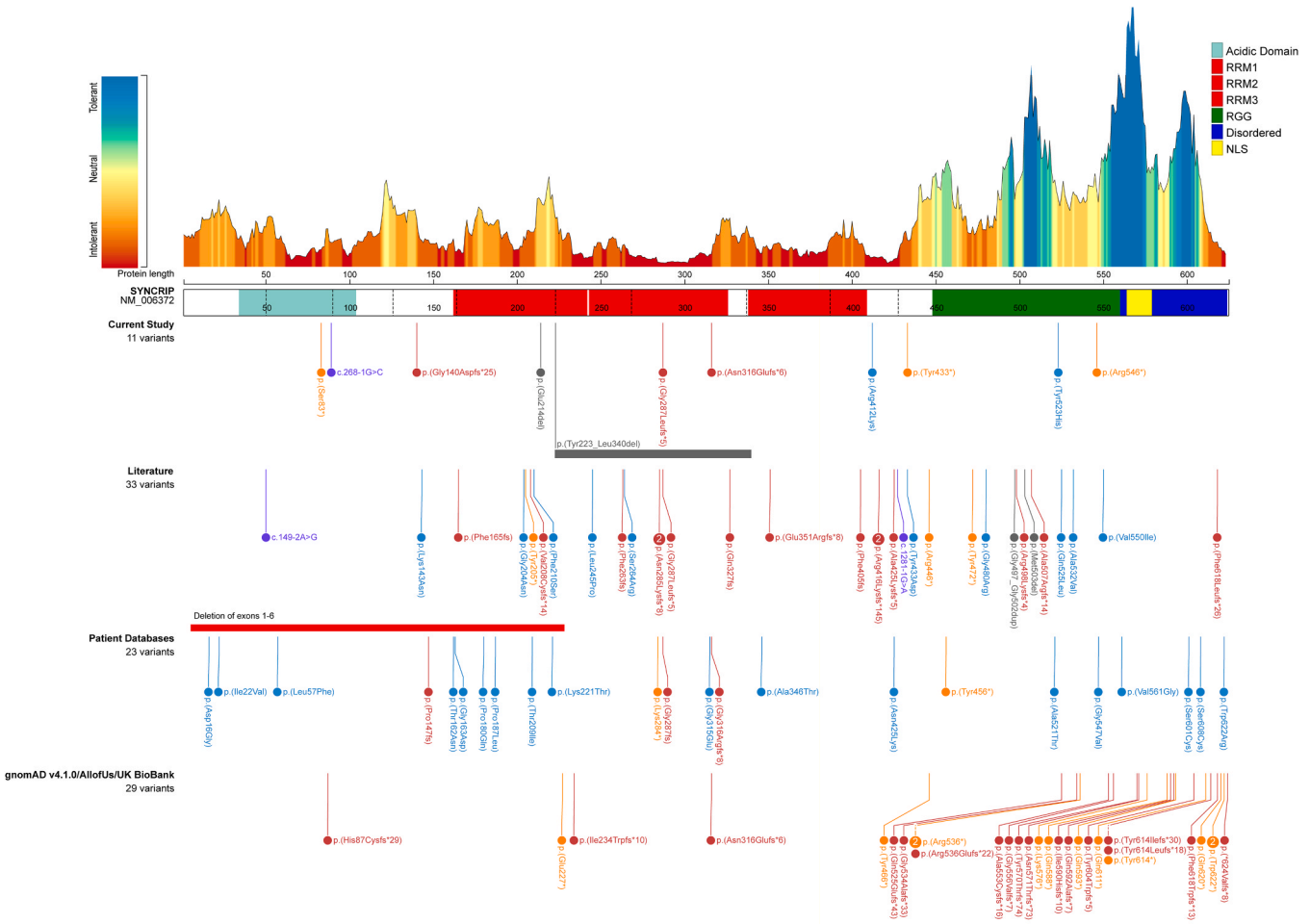

Fig. 2. Variants observed in *SYNCRIP* in our study, the literature, patient databases, and large population studies. Frameshifts are shown in red, nonsense variants are shown in orange, splice variants are shown in purple, missense variants are shown in blue, and indels are shown in grey. The top panel shows the intolerance metrics from MetaDome. RRM: RNA Recognition Motif, RGG: RGG Box, NLS: bipartite nuclear localization signal.

(Fig. 2). *SYNCRIP* is predicted to be sensitive to loss-of-function variants (pLI = 1) and missense variation (Z = 4.84).

Eight missense and one in-frame single amino acid deletion are in the RNA recognition motif 1 domain (RRM1, amino acids 162–241), five missense variants are in the RRM2 domain (amino acids 243–325), and one missense variant is in the RRM3 domain (amino acids 338–408). *In silico* tools predict these missense variants to be damaging but no functional studies have been done to definitively show pathogenicity. These domains are sensitive to variation (Fig. 1). Thus, it is likely that RNA-binding is disrupted in these patients. Fifteen variants, including missense and possibly protein elongating or truncating, are located in the disordered region of SYNCRIP. This region partially interacts with SMN and includes the bipartite nuclear localization signal.

One of the splicing variants (c.268–1 G>C, Patient 2) is expected to result in NMD, while two are predicted to result in use of a cryptic splice site and may result in in-frame-protein products (c.149–2 A>G and c.1281–1 G>A). Three missense variants in exon 11 (p.(Val550Ile), p.(Ala532Val), and p.(Ser608Cys)) are also predicted to result in possible splice donor site loss and use of a cryptic splice site resulting in an out of frame protein. As these are in the final exon, it is unclear if these would be subjected to NMD or result in altered proteins.

While haploinsufficiency is likely for SYNCRIP-Related Neurodevelopmental Disorder, only 19 of the 30 predicted loss-of-function variants are strongly predicted to undergo NMD. Eleven may undergo NMD or result in a truncated protein, while one is predicted to elongate the protein by 19 amino acids or could be degraded by non-stop RNA decay. For those that may not undergo NMD, it is plausible that the protein products may result in gain-of-function or decreased/loss of function. Functional studies will be necessary to determine the pathogenicity of these types of variants in *SYNCRIP*.

3.4. *Phenotypic spectrum of SYNCRIP-Related Neurodevelopmental Disorder*

Phenotypes of individual patients are outlined in supplementary tables. Twenty-six individuals are male and 15 individuals are female. The average age for males was 9.5 years, for females was 9.4 years, and overall was 9.6 years. Variants were grouped into four categories: likely gene disruptive/loss-of-function (LGD/LOF), missense (all with CADD scores of at least 20), in-frame deletion/duplications including both single amino acid changes and larger alterations, and variants predicted to escape NMD. Missense variants in exon 11 were included in the missense category. Splice variants were assessed by SpliceVault to determine if they were predicted to result in loss-of-function or in-frame exonic changes.

For those with information available, 43 of 47 (91.5 %) individuals from the literature, patient databases, and our novel cohort reportedly have DD/ID. Four of these had mild DD/ID and three had borderline DD/ID. The presence of or severity of DD/ID did not correlate with variant type. Seventy-seven percent (n = 30/39) had speech delays, some of which were complicated by dystharia or tongue and lip tie. Presence of speech delay did not correlate with variant type. Four

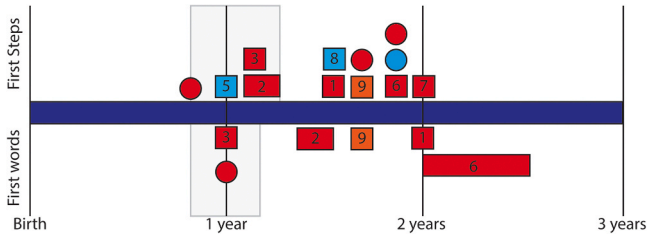

Fig. 3. Age of meeting developmental milestones for those who had information available. Average ages are shown in grey boxes. Circles indicate patients from literature or databases. Squares are labeled with the novel patient number, and ranges, when indicated, are represented by length of the box. Blue: missense and in-frame variants, red: frameshift variants, orange: nonsense variant.

patients are nonverbal, one only after regression of skills (Patient 4). For those with language and who reported age of first words, the average age of first words was ~15.8 months (Fig. 3). Cognitive and speech regression was observed in six individuals (15 %, n = 40), four of which have variants predicted to escape NMD. Prior to Bonferroni correction, those with variants predicted to escape NMD had significantly more individuals with regression reported.

Of those with information available, 57 % (n = 21/37) had motor delays, with no difference between variant types. One individual (Patient 4) is non ambulatory after regression of skills, one is reportedly "slow moving" (Patient 1), six have altered gait or ataxia, two are noted to be clumsy or have poor balance, and one each are dystonic, have hyperkinetic movements and spastic diplegia, have choreoathetoid movements, or have bradykinesia. For those with clinical information available (n = 11) the average age of first steps was slightly delayed at ~16.9 months (Fig. 3). Hypotonia is common, observed in 41.2 % of patients (n = 14/34). While not significant after Bonferroni correction, those with LFD/LOF variants trended towards being more likely to be hypotonic.

Behavioral differences are common in SYNCRIP-RNDD. Over half (63 %, n = 29/46) have ASD or autistic features, with similar rates across all variant types. ADHD or inattention was reported in 27.7 % (n = 10/36). Sleep disturbances were present in 20.5 % (n = 10/36). Aggressive behaviors and/or abnormal tantrum behavior were reported in eight individuals, although limited data was available. One patient (Patient 5) also had no sense of danger while four had anxiety and one was depressive. One individual was also noted to have screaming and bouts of laughter. Some patients exhibited drooling.

Structural brain anomalies are prevalent but inconsistent. Of the twenty-six individuals who have had brain imaging, thirteen individuals had positive brain MRIs (50 %). Two individuals had Chiari malformations, four had ventriculomegaly-like features such as widening of CSF spaces and ventricles, one each had abnormal periventricular signal, periventricular nodular heterotopia, white matter loss, an arachnoid cyst, thin corpus callosum, malrotation of the hippocampus, and nonspecific punctate changes in the basal ganglia and subcortical white matter. Seizures were present or suspected in 35.7 % of individuals (n = 15/42). No significant differences were observed by variant type.

Dysmorphic features are common (n = 26/39, 67.9 %) but inconsistent. Five individuals have abnormal ears, four have epicanthus, four have macrocephaly, three have a thin upper lip, three have deep-set eyes, and two each have down slanting or up slanting palpebral fissures. No significant differences were observed, although all three individuals with predicted in-frame alterations and clinical information available had some features reported. Physical anomalies were sparingly reported, including 2–3 toe syndactyly and mottling of toes (Patient 2), thin skin (Patient 4), prominent teeth (Patient 7), bradycardia (Patient 7), cleft palate (SCV004027820.1), microcephaly (407065) or macrocephaly, hypopigmentation (407065), short neck (DDD4K.01634), cervical segmentation defect (DDD4K.01634), and finger anomalies.

Several phenotypes were observed in small numbers of patients. Vision impairment was reported in about one quarter of individuals, including hyperopia (n = 4), astigmatism (n = 3), myopia (n = 3), and cerebral visual impairment (n = 1). Mild to moderate hearing loss was observed in three patients. Genitourinary abnormalities are rare but include diagnosed or suspected neurogenic bladder (n = 2), cryptorchidism (n = 2), retractile testes (n = 1), and nocturnal enuresis (n = 1). Kidney anomalies included metacystic kidneys (n = 2) and unilateral renal agenesis (n = 1). Feeding difficulties, including G-tube feeding, were reported in four individuals.

3.5. *Evidence for haploinsufficiency*

Skin biopsies were collected for two individuals (Patients 3 and 9). From the generated fibroblasts, the protein levels of SYNCRIP/hnRNPQ were assessed (Fig. 4). Both patient cell lines, one of which carries a frameshift variant and one of which carries a nonsense variant in the final exon, have similarly decreased levels of SYNCRIP/hnRNPQ compared to the control. This suggests that at least some nonsense variants in the final exon do result in nonsense mediated decay as opposed to truncation. Notably, both patient cell lines have about 70 % of the SYNCRIP/hnRNPQ levels compared to controls. As hnRNPs are known to autoregulate, it's likely that such a mechanism is increasing the expression of SYNCRIP/hnRNPQ from the remaining allele. Interestingly, a cell line from an individual with HNRNPU-RNDD had increased SYNCRIP/hnRNPQ levels (data not shown), suggesting they may have a functional relationship. A relationship between protein levels of SYNCRIP/hnRNPQ and hnRNPU has been observed in organoids derived from individuals with HNRNPU-Related Neurodevelopmental Disorder [65].

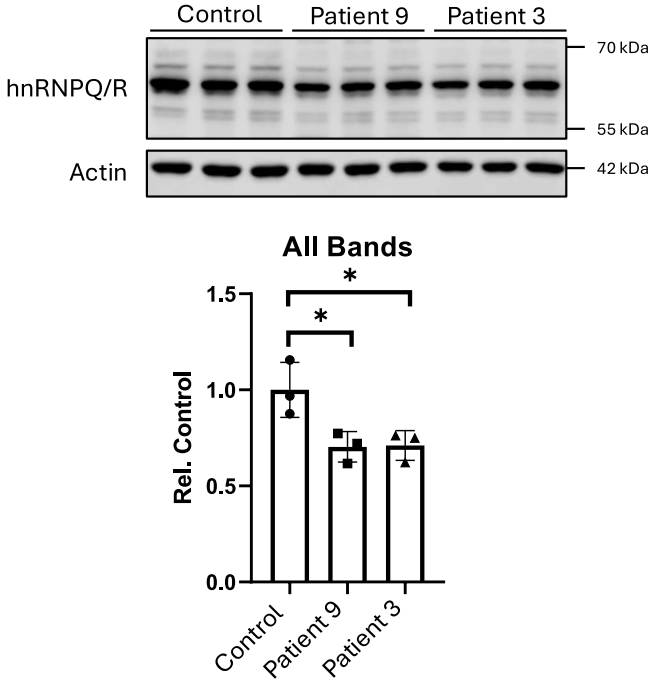

Fig. 4. Protein quantification from two patient fibroblast lines (Patient 3 and Patient 7). Patient 3 has a likely *de novo* (possibly mosaic in his father) frameshift variant (NM_006372.4:c.415_418dup, p.(Gly140Aspfs*25)). Patient 7 has a *de novo* nonsense variant in the last exon of *SYNCRIP* (NM_006372.4: c.1299 T>G, p.(Tyr433*)). Protein levels, compared to a control, are reduced by about 30 % in SYNCRIP-RNDD patients. This suggests that at least some nonsense variants in the last exon of the gene are subjected to nonsense mediated decay.

4. Discussion

This case series discusses individuals with predicted damaging variants resulting in SYNCRIP/HNRNPQ-Related Neurodevelopmental Disorder (SYNCRIP-RNDD). *SYNCRIP* has been implicated in DD/ID and ASD in several large cohort studies, as well as a smaller group of more deeply phenotyped patients [15,25]. Overall, the phenotypic findings of SYNCRIP-NDD include mild to moderate DD/ID, speech and language delay, behavioral differences such as ASD and ADHD, mild motor delays, hypotonia, variable structural brain anomalies, seizures, and inconsistent dysmorphic facies. In addition to phenotypic variability, the spectrum is also broad in terms of degree of involvement. These clinical features are consistent with previous reports as well as with the notion that loss of *SYNCRIP* contributes to NDD features in proximal 6q deletions [15,25,49].

The HNRNP-RNDDs have been observed to have overlapping clinical features. All consist of DD/ID of varying severity, speech and language delay, behavioral differences such as ASD and ADHD, and brain imaging anomalies. Many of the HNRNP-RNDDs have seizures as well, particulary individuals with HNRNPU-RNDD. In particular, SYNCRIP-RNDD has been reported to have the most overlap with HNRNPR-RNDD, and the SYNCRIP/hnRNPQ and hnRNPR proteins are highly homologous [15]. While phenotypic information is limited for HNRNPR-RNDD as well, those with SYNCRIP-RNDD appear to have similar but less severe features [12]. While individuals with SYNCRIP-RNDD have borderline to moderate DD/ID, many with HNRNPR-RNDD have moderate to severe DD/ID. The structural brain anomalies observed in HNRNPR-RNDD are more consistent. Behaviorally, both disorders consist of ADHD in some patients, but ASD appears to be more common with variation in *SYNCRIP*. Both groups have abnormal ears, and some features observed in HNRNPR-RNDD probands are seen in few SYNCRIP-RNDD probands, including a short neck, micrognathia, abnormal nasal bridges, and up slanted palpebral fissures. Some SYNCRIP-RNDD patients have skeletal and/or hand and feet anomalies, but these occur less often and of less severity compared to HNRNPR-RNDD. Further expansion of both disorders' phenotypes, as well as the other HNRNP-RNDDs, is necessary to understand the full extent of this overlap. Remarkably, Patient 9, who did not consent for photos, shares many facial features typically seen in females with HNRNPH2-RNDD, highlighting additional possible phenotypic and molecular overlap among these disorders.

In total, we reviewed ten novel cases, one case that had been previously published with limited information, 33 cases from the literature, and 23 cases from patient databases. The vast majority of cases occurred *de novo*, although inherited variants were reported as well. Several variants expected to be pathogenic were observed in gnomAD v4.1.0, *All of Us*, and/or the UK Biobank. The latter two databases include affected individuals, so the variants identified may be in persons with SYNCRIP-RNDD. However, gnomAD is typically adult individuals without pediatric onset disease, although there is no "non-neuro" database for the most recent version. Many of the variants in these databases were present in the last exon, in which frameshift variants would be predicted to escape NMD. Future functional work to determine the molecular impact of such variants will be necessary. It is also possible that less severely affected individuals are present in population studies, representing either incomplete penetrance and/or variable expressivity with subclinical phenotypes. Several of our probands have borderline ID, suggesting adults with milder neurodevelopmental phenotypes could easily be present in population cohorts.

Genotype-phenotype relationships have yet to be described in SYNCRIP-RNDD. Here, we did not see any significant differences between loss of function and missense variants, although the one patient carrying a single amino acid deletion does have unique features. Missense variants, particularly in the RRM domains, may result in decreased function and a similar phenotypic presentation to loss of function variants. It is unclear if Patient 4 represents an expansion of the SYNCRIP-RNDD phenotype. His early development was consistent with SYNCRIP-RNDD with developmental delays. However, after undergoing surgery with anesthesia he had rapid regression of skills resulting in being nonverbal and non-ambulatory. Anecdotally, individuals with HNRNP-RNDDs appear to metabolize drugs faster, and he is not the only individual who has had complications with anesthesia -although other individuals have had anesthesia and had no issues. His mother was reportedly told he has a juvenile-ALS-like clinical picture. As the hnRNPs have been implicated in ALS, mostly through changes in expression or pathogenic variants in the *FUS* and *TARDPBP* genes, this suggests that disruption of hnRNPs in the context of a *SYNCRIP* variant may result in an altered, more severe phenotype that may depend on the nature of the variant. He has the only single amino acid deletion within an RRM to date, suggesting a possible different disease mechanism. It is also possible he may have a secondary diagnosis that is unknown, although no other variants of interest were identified by exome sequencing.

Based on the pLI score and the identified variants, it has been assumed that SYNCRIP-RNDD is the result of loss of function of SYNCRIP/hnRNPQ. For the two patients with available fibroblasts, one with a frameshift variant predicted to undergo NMD and one with a nonsense variant in the last exon, SYNCRIP/hnRNPQ levels were decreased, although not to half the amount. As hnRNPs have complex regulation of themselves and related genes, this may represent a compensation mechanism. Further studies will be necessary to see how other variants impact *SYNCRIP* expression and to determine if there are multiple disease mechanisms. Here, we show that at least a subset of individuals with SYNCRIP-RNDD are haploinsufficient for the *SYNCRIP* gene.

5. Conclusions

This case series highlights the phenotypic presentations of individuals with SYNCRIP-RNDD. With these recent unpublished cases, we have added several phenotypic features such as neurogenic bladder, potential developmental regression, and absence seizures that were not previously discussed in other reports. We have expanded the phenotype and provided guidance towards future work regarding functional characterization of variants. Furthermore, we have observed possible incomplete penetrance of *SYNCRIP* variants. Finally, we have shown that haploinsufficiency, with a possible autoregulatory mechanism to increase *SYNCRIP* expression, is a likely pathomechanism of SYNCRIP-RNDD.

Ethics statement

All subjects gave their informed consent for inclusion before they participated in the study. The study was conducted in accordance with the Declaration of Helsinki, and the protocol was approved by both the Columbia University and University of Calgary Institutional Review Boards (IRBs).

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Declaration of Competing Interest

Authors have nothing to disclose

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.rare.2024.100052.

Data availability

No data was used for the research described in the article.

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