Mutations in *NRXN1* and *NRXN2* in a patient with early-onset epileptic encephalopathy and respiratory depression

Anne M. Rochtus,[1,2,3] Sara Trowbridge,[3,4] Richard D. Goldstein,[2,5] Beth Rosen Sheidley,[1,3] Sanjay P. Prabhu,[2,6] Robin Haynes,[2,7] Hannah C. Kinney,[2,7] and Annapurna H. Poduri[1,2,3,4,8]

[1]Epilepsy Genetics Program, Department of Neurology, [2]Robert's Program on Sudden Death in Pediatrics, [3]Department of Neurology, Boston Children's Hospital, Boston, Massachusetts 02115, USA; [4]Department of Neurology, Harvard Medical School, Boston, Massachusetts 02115, USA; [5]Department of Pediatrics, [6]Department of Radiology, [7]Department of Pathology, Boston Children's Hospital and Harvard Medical School, Boston, Massachusetts 02115, USA; [8]Broad Institute, Cambridge, Massachusetts 02142, USA

Abstract Early infantile epileptic encephalopathy (EIEE) is a severe disorder associated with epilepsy, developmental delay and intellectual disability, and in some cases premature mortality. We report the case of a female infant with EIEE and strikingly suppressed respiratory dysfunction that led to death. Postmortem research evaluation revealed hypoplasia of the arcuate nucleus of the medulla, a candidate region for respiratory regulation. Genetic evaluation revealed heterozygous variants in the related genes *NRXN1* (c.2686C>T, p.Arg896Trp) and *NRXN2* (c.3176G>A, p.Arg1059Gln), one inherited from the mother with family history of sudden infant death syndrome (SIDS) and one from the father with family history of febrile seizures. Although there are no previous reports with the digenic combination of *NRXN1* and *NRXN2* variants, patients with biallelic loss of *NRXN1* in humans and double neurexin 1α/2α knockout mice have severe breathing abnormalities, corresponding to the respiratory phenotype of our patient. These observations and the known interaction between the *NRXN1* and *NRXN2* proteins lead us to hypothesize that digenic variants in *NRXN1* and *NRXN2* contributed to the phenotype of EIEE, arcuate nucleus hypoplasia, respiratory failure, and death.

[Supplemental material is available for this article.]

Corresponding author:
annapurna.poduri@childrens
.harvard.edu

INTRODUCTION

The developmental and epileptic encephalopathy (DEE) syndromes that present in infancy include Ohtahara syndrome, or early infantile epileptic encephalopathy (EIEE), which is characterized by a burst suppression pattern on electroencephalogram (EEG) and a generally poor prognosis, with high mortality rates in infancy (Beal et al. 2012). Anecdotally, we have observed that EIEE is associated with dysfunctional regulation of respiration, which has also been described in the related epileptic encephalopathy West syndrome (Jansen et al. 2013). The anatomic basis of respiratory dysfunction in such cases has not been studied in detail, and hypotheses include the role of central nervous system depression that may be exacerbated by medications given for seizure control as well as dysregulation of the normal

processes of developmental maturation of respiration. Neurexins are cell adhesion proteins that play a role in synaptic transmission through interaction with neuroligins. Deletions within *NRXN1* have been associated with a range of neurodevelopmental disorders including intellectual disability, developmental delay, speech and language delay, autism spectrum disorders, schizophrenia, and epilepsy (Curran et al. 2013; Møller et al. 2013; Lal et al. 2015). Heterozygous loss of function of *NRXN1* has been associated with susceptibility to generalized epilepsy (Olson et al. 2014; Lal et al. 2015). Interestingly, epilepsy is a more consistent feature in patients with more carboxy-terminal deletions, including the β-isoform (Schaaf et al. 2012; Dabell et al. 2013). Homozygous disruption of *NRXN1* in humans causes a severe Pitt–Hopkins-like syndrome including early-onset epilepsy, breathing difficulties, severe intellectual disability, absent speech, stereotypies, and autistic features (Zweier et al. 2009; Harrison et al. 2011; Duong et al. 2012; Béna et al. 2013; Imitola et al. 2014). Variants and truncations in *NRXN2*, although less frequently described, have been associated with autism spectrum disorder and language delay (Gauthier et al. 2011). Deletion of the *Nrxn2α* gene in mice can replicate some of the core symptoms of autism (Dachtler et al. 2014). Here we present the case of a female infant with EIEE with striking respiratory dysfunction, particularly during sleep, who was found to have heterozygous variants in both *NRXN1* and *NRXN2*, one inherited from each parent. Although premortem neuroimaging had been assessed as normal, extensive postmortem neuroimaging and neuropathology were performed to evaluate the regions that contribute to regulation of respiratory function and that are thought to contribute to premature death in conditions such as sudden infant death syndrome (SIDS), sudden unexpected death in childhood with febrile seizures, and sudden unexpected death in epilepsy patients (SUDEP).

RESULTS

Clinical Presentation and Family History

The patient was an infant girl who initially presented at 8 d of life for poor feeding, lethargy, and weight loss. She was born to a 25-yr-old G2P2 mother, who was on levothyroxine and insulin for hypothyroidism and gestational diabetes. Labor was induced at 38 weeks of gestation for decreased fetal movement and concerning nonstress tests, but the patient emerged vigorous with Apgar scores of 8 at 1 and 5 min. Her examination was notable for a weak cry, and laryngoscopy revealed supraglottic edema. She was discharged but continued to have difficulties with breastfeeding and became increasingly lethargic. She presented at 8 d of life with significant lethargy and hypertonicity of the extremities. Initial diagnostic evaluation included MRI of the brain and spine, which were considered normal. Video-EEG monitoring was performed to evaluate whether apneic episodes were seizures. Although those episodes were not associated with EEG seizures, the study revealed a markedly abnormal background with a burst suppression pattern, as well as multiple focal seizures arising from the midline and bilateral parietal regions that manifested clinically as bilateral tonic arm extension. Based on her seizures, encephalopathy, and EEG findings, she was diagnosed with EIEE. She was treated with multiple antiepileptic drugs (AEDs), including phenobarbital, levetiracetam, zonisamide, and intermittent diazepam. Her clinical seizure frequency declined, but she continued to have frequent subclinical seizures with continued burst suppression pattern in the background. She had empiric trials of pyridoxine, pyridoxal-5-phosphate, folic acid, and biotin, with no effects on her clinical status or EEG. She also had a comprehensive metabolic evaluation (including serum lactate, pyruvate, amino acids, and cerebrospinal fluid amino acids), which did not identify an etiology for her presentation.

The patient's clinical course was additionally marked by an irregular breathing pattern, with prolonged periods of apnea associated with desaturation. These episodes did not
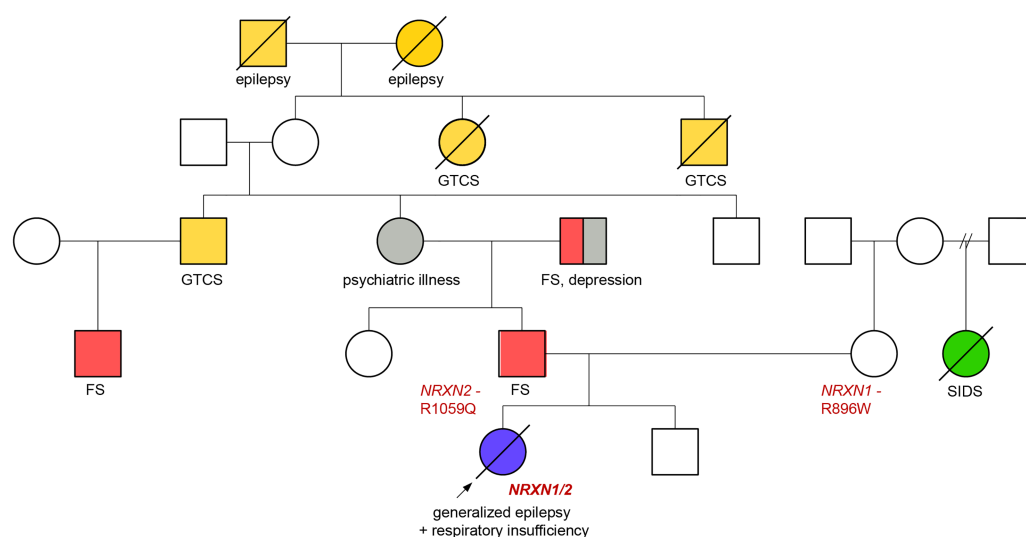
Figure 1. Pedigree of the family. (FS) Febrile seizures in childhood, (GTCS) generalized tonic–clonic seizures, (SIDS) sudden infant death syndrome.

correlate with EEG changes and were thus not thought to be due to seizure activity; of note, they often occurred during clinical sleep periods. Over the hospital course, her respiratory support requirement escalated from none initially, to supplemental oxygen via nasal cannula, to noninvasive positive pressure support (CPAP and BiPAP), and finally to intubation on day of life 38. Attempts were made to minimize the use of as-needed diazepam for clusters of clinical seizures in the event that this intervention was affecting her respiratory reserve, but even in the absence of additional diazepam respiratory effort was compromised. Given the patient's escalating respiratory support requirements and overall grim prognosis due to her EIEE diagnosis and persistent burst suppression pattern on EEG, a multidisciplinary and family meeting was held on day of life 43, at which point the decision was made to redirect care, and she died on day of life 49 following extubation. The patient's parents requested to donate her brain to research; postmortem neuroimaging and neuropathology studies were performed after consent for autopsy and enrollment into studies approved by the Boston Children's Hospital Institutional Review Board.

The patient's family history was notable for SIDS (mother's half-sister), as well as generalized tonic–clonic seizure and/or febrile seizures in multiple family members across three generations in her paternal grandmother's family. The patient's father and her father's sister both had multiple febrile seizures in childhood and were developmentally normal (Fig. 1).

Pre- and Postmortem Phenotypic Analyses
Neuroimaging

Conventional premortem neuroimaging performed at 10 d of age had not revealed a significant abnormality apart from mildly increased T2 signal intensity in the bilateral dentate nuclei of the cerebellar hemispheres. Notably, coronal imaging detailing the hippocampus was not performed as it is not part of the routine neonatal imaging protocol.

High-resolution postmortem imaging performed on a research basis revealed asymmetry of the hippocampal formations, with a globular shape and slightly larger size of the left hippocampus (Fig. 2A). Also, note was made of excessive folding and hyperconvolution of the dentate gyrus bilaterally, lending a serrated appearance to the dentate gyrus, more
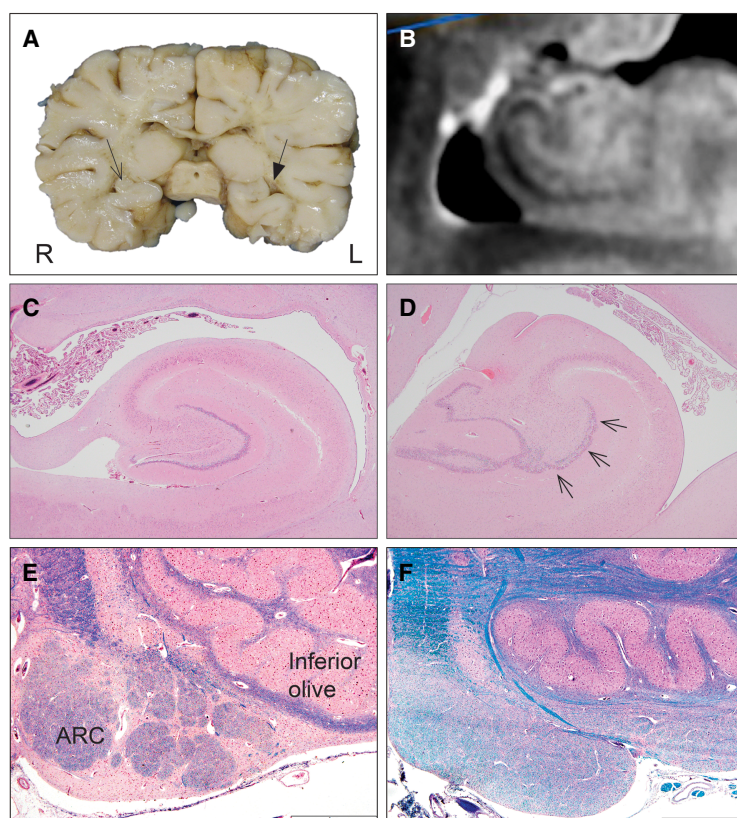
Figure 2. Postmortem neuroanatomy and neuropathology. (*A*) Macroscopic abnormalities of the brain with a globular shape and slightly larger size of the left hippocampus (filled triangle arrow). (*B*) High-resolution T2 postmortem magnetic resonance imaging of the right hippocampus. (*C*) Control hippocampus. H&E staining, 2×. (*D*) Pathology of the patient's left hippocampus demonstrating focal granule cell bilamination in the dentate gyrus (small arrows). H&E staining, 2×. (*E*) Control arcuate nucleus (ARC, arrows) in clusters along the pyramid at the ventral surface of the medulla. H&E, 4×. (*F*) Absence of ARC clusters in the brainstem of the patient. LFB staining, 4×.

appreciably on the right (Fig. 2B,C). Close examination of the brainstem showed small inferior olivary nuclei and hypoplasia of the arcuate nucleus (Fig. 2E).

Neuropathological examination revealed bilateral maldevelopment of cortical and hippocampal folding with focal cortical dysplasia and granule cell dispersion in the dentate gyrus, with partial bilamination (right hippocampus shown in Fig. 2B and left hippocampus in Fig. 2C, with left dentate gyrus bilamination in Fig. 2C). In addition to the cortical abnormalities, there was anomalous development of the olivo-arcuate system in the brainstem, with significant hypoplasia of the arcuate nucleus along the ventral medullary surface (Fig. 2E). There was mild gliosis of the reticular formation of the medulla, including the paragigantocellularis lateralis, the putative central rhythm generator in the rostral medulla of experimental animals homologous to the pre-Bötzinger complex. Other cardiorespiratory-related nuclei throughout the brainstem (e.g., the nucleus of the solitary tract) were unremarkable.

Genomic Analyses

The patient had been evaluated for a genetic diagnosis with a range of investigations including initially commercial chromosomal microarray and single-gene sequencing of

Table 1. Variant table

Gene	Variant	Chr	HGVS DNA Ref.	HGVS protein Ref.	Variant type	Predicted effect	dbSNP ID	Genotype	ClinVar ID
NRXN1	c.2686C>T (p.Arg896Trp)	2	NM_004801	NP_004792	Missense	Substitution	rs79605277	Heterozygous	SCV000241889.13
NRXN2	c.3176G>A (p.Arg1059Gln)	11	NM_138732	NP_620060	Missense	Substitution	rs777033569	Heterozygous	SCV000863537

(Chr) Chromosome, (HGVS) Human Genome Variation Society.

PHOX2B, associated with congenital central hypoventilation syndrome (CCHS), which were unrevealing. A comprehensive epilepsy gene panel, including deletion/duplication analysis, revealed variants of unknown significance in *GAMT* (NM_00156.4, c.79T>C, p.Tyr27His), *MBD5* (NM_018328.4, c.2000T>G, p.Leu667Trp), and *NRXN1* (NM_004801.4, c.2686C>T, p.Arg896Trp), all of which were inherited. Because these findings could not fully explain the EIEE and respiratory phenotype, whole-exome sequencing (WES) of the patient and her parents was performed and revealed an additional variant in *NRXN2* (NM_138732.2, c.3176G>A, p.Arg1059Gln). We did not find de novo, homozygous recessive, or compound heterozygous variants that could definitively explain the phenotype of our patient (Supplemental data). In addition, we did not find variants in phenotype-related genes or from a large candidate gene list that could explain the phenotype. The variant in *NRXN1* was shown to be maternally inherited and located in exon 15, in the carboxyl terminus of the α-neurexin form. The *NRXN2* variant was paternally inherited and located in exon 16, in the carboxyl terminus of the α-neurexin form (Supplemental Fig. S1). The information of the variants is shown in Table 1. The allele frequency and pathogenicity prediction of the variants are shown in Table 2. Both variants are rare/absent in the general population and are predicted to be deleterious according to different tools of pathogenicity prediction. In addition, both genes are under functional constraint (Lek et al. 2016). Both variants have been classified using the ACMG guidelines (Richards et al. 2015) as variants of unknown significance.

An in silico analysis within Decipher revealed 19 patients with *NRXN1* and seizures. One patient with a copy-number variation (CNV) loss also had breathing dysregulation, generalized tonic–clonic seizures, and severe developmental delay (ID: 266445). There were no patients with *NRXN2* and seizures in Decipher. About 13/18 patients with variants or CNVs in *NRXN2* had intellectual disability or neurodevelopmental delay and one patient presented with asthma.

Table 2. Functional prediction and conservation scores of the two variants

Gene	Variant	AF (gnomAD)	SIFT	PP2-HDIV	MT	CADD	z-score	pLI	GERP-RS	ClinVar
NRXN1	c.2686C>T (p.Arg896Trp)	0	D	D	DC	26.5	3.02	1	4.52	VUS
NRXN2	c.3176G>A (p.Arg1059Gln)	1.22×10^5	D	D	DC	29.7	5.81	1	4.13	N/A

(AF) Allele frequency, (CADD) combined annotation-dependent depletion, (D) deleterious, (DC) disease causing, (GERP) Genomic Evolutionary Rate Profiling, (MT) MutationTaster, (N/A) not available, (pLI) loss-of-function constraint metric, (PP-2) PolyPhen-2, (SIFT) Sorting Intolerant from Tolerant, (VUS) variant of uncertain significance, (z-score) missense constraint metric.

DISCUSSION

We present the case of a female infant with EIEE and strikingly suppressed respiratory drive during sleep. Although it is not uncommon for infants with severe epilepsy to require intubation when having many seizures and receiving multiple AEDs, respiratory failure occurred in our patient in the setting of only a modest combination of medications, and persistent respiratory failure could not be explained by the number of seizures nor the degree of medication that was administered to treat seizures. The premortem observations of deficient respiratory drive and reserve correlate with our postmortem observations—on neuropathology and neuroimaging—of near-complete absence of the arcuate nucleus. The diagnosis of EIEE itself warrants a thorough evaluation for etiology, including genetic etiology with a focus on epilepsy-related genes (Olson et al. 2017). In addition, the profound respiratory phenotype, in combination with the arcuate hypoplasia and temporal lobe abnormalities noted postmortem, prompted additional research analysis with WES.

The combination of premortem diagnosis and a coordinated postmortem neuroradiological and neuropathological assessment led to a more detailed phenotype that incorporated previously unrecognized abnormalities of not only the cerebral cortex and hippocampi but also the brainstem in the form of almost complete absence of the arcuate nucleus. Our group has shown previously that the arcuate nucleus is cytologically and spatially homologous to the ventral respiratory chemosensitive zones in cats on the ventral medullary surface (Filiano et al. 1990). We also reported severe arcuate hypoplasia in ~5% of SIDS cases (Filiano and Kinney 1992; Kinney et al. 2016), as well as binding defects in the arcuate nucleus in SIDS cases to neurotransmitters implicated in ventral chemosensory control—for example, muscarinic cholinergic receptors (Kinney et al. 1995) and serotonergic receptors (Panigrahy et al. 2000). Interestingly, the arcuate nucleus has been previously implicated in the respiratory function and chemoreception in a few case reports of CCHS (Folgering et al. 1979; Tomycz et al. 2010). Hypoplasia of the arcuate nucleus and neurochemical abnormalities in SIDS (Filiano and Kinney 1992; Kinney et al. 1995; Panigrahy et al. 1997, 2000) raise the possibility that at least a subset of SIDS cases have impaired central chemosensitivity that may contribute to respiratory dysfunction and death. Recent studies in adults have underscored the possible role of the arcuate nucleus at the ventral medullary surface as critical for central chemosensitivity (Benarroch et al. 2007) in human adult disorders. Besides arcuate nucleus hypoplasia, the neuropathological assessment of our case also showed hippocampal asymmetry and dentate gyrus anomalies. We have reported these findings previously in toddlers with sudden death and febrile seizures (Kinney et al. 2016), raising the question of whether these anatomical defects, thought to be developmental, contribute to sudden death through an interaction with respiratory control mechanisms, although the precise mechanisms have yet to be elucidated.

Detailed postmortem neuropathological assessments of patients with EIEE are limited to a few case reports, including one from our group describing a patient with a pathogenic variant in *SCN2A* (Touma et al. 2013). We had hypothesized that the profound respiratory dysfunction in our patient was due to an independent defect in the gene *PHOX2B*, but this proved not to be the case. We did not identify a genetic cause of the patient's epilepsy, suspected genetic, with the first tier of clinical testing with CMA and epilepsy panel. However, the nature of the epileptic encephalopathy and the positive family history on both sides of the family suggested that a combination of genetic factors may have together contributed to the patient's phenotypes. Having identified two disease-relevant variants in two related genes, *NRXN1* and *NRXN2*, we hypothesize a digenic process in which neither variant is sufficient to produce severe disease on its own but in combination the two together could have led to our patient's phenotype. Digenic inheritance is a hypothesized form of complex

genetic disease, only a small number of conditions have been reported with digenic inheritance—for example, some forms of deafness, long QT syndrome, and Bardet–Biedl syndrome (Schäffer 2013). An operational definition by Schäffer says: "inheritance is digenic when the variant genotypes at two loci explain the phenotypes of a patient more clearly than the genotype at one locus alone" (Schäffer 2013). The family history of SIDS on the maternal side and febrile seizures on the paternal side suggest that there may be some contribution of the neurexin-related variants in other phenotypes. Unfortunately, DNA is not available from additional family members, including the child who died of SIDS, to further explore this hypothesis within the pedigree.

Neurexins are neuronal adhesion molecules located in the presynaptic terminal, where they interact with postsynaptic neuroligins to form a trans-synaptic complex required for efficient neurotransmission in the brain. In mammals, three neurexin genes (*NRXN1*, *NRXN2*, and *NRXN3*) have been described. They are mainly localized at the presynaptic terminal and form heterophilic interactions with neuroligins, which are localized to the postsynaptic compartment. Thus, the neurexin–neuroligin complex forms a trans-synaptic adhesion complex. Each neurexin gene encodes a long α-neurexin isoform and a short β-neurexin isoform. In addition to the two major isoforms, there are five conserved alternative splice sites in α-neurexins (splice site [SS]#1 to SS#5) and two in β-neurexins (SS#4 and SS#5), whereby they can be transcribed in thousands of alternatively spliced isoforms, contributing to distinct structural domains and variability (Reissner et al. 2013; Südhof 2017). α-Neurexins contain six LNS (laminin-neurexin-sex hormone binding globulin) domains with three epidermal growth factor (EGF)-like domains interspersed. β-Neurexins are identical to the carboxyl terminus of α-neurexins starting from the last LNS domain but have a unique amino-terminal stretch of 37 histidine-rich residues. The LNS domains are thought to behave like glycan-binding lectins. The variants of our patient in *NRXN1* and *NRXN2* are located in LNS4 and LNS5, respectively. A general function of the LNS domains has not been demonstrated so far, but, for example, dystroglycan requires a specific glycosylation to bind to LNS4-5 (Wizemann et al. 2003). In addition, the variants are located nearby SS#3. Although we do not have functional studies, these findings might support the possibility that the variants influence splicing and the transcription alternative splice isoforms.

To date, only six case reports of biallelic loss of *NRXN1* in humans have been described (Table 3; Zweier et al. 2009; Harrison et al. 2011; Duong et al. 2012). Patients with biallelic loss of *NRXN1* have a variable neurodevelopmental phenotype, presenting with epilepsy, breathing abnormalities, speech delay, and abnormal sleep–wake cycle. Some of the reported patients with biallelic loss of *NRXN1* variants have severe breathing abnormalities. Although highly similar, our patient had a more severe phenotype than the patients with biallelic loss of *NRXN1*. She presented with arcuate nucleus hypoplasia, hippocampal asymmetry, and dentate gyrus anomalies, raising the question, "Did these anomalies contribute to the death of our patient?" Thus, it seems plausible that biallelic loss of *NRXN1* in humans could lead to a fully penetrant, severe neurodevelopmental phenotype, whereas the heterozygous deletion likely represents a susceptibility factor for variable cognitive, neurological, and psychiatric disorders (Béna et al. 2013). Functional studies in mice revealed that deletion of α-neurexins did not impair prenatal viability but markedly compromised postnatal survival. Triple-knockout mice died on the first day and most double-knockout mice died within the first week. Remarkably, combined Nrxn1a$^{-/-}$ and Nrxn2a$^{-/-}$ and triple-knockout mice had the same level of breathing difficulties. In addition, they showed that the irregular respiratory rhythm of these mice was an intrinsic property of the brainstem (Missler et al. 2003; Etherton et al. 2009).

We have assessed the findings in our patient in the context of patients with biallelic loss of *NRXN1* and studies in mice involving *Nrxn1* and *Nrxn2*. The reported studies provide some evidence to support the assertion that, in principle, digenic pathogenic variants in

Table 3. Clinical characteristics of patients with biallelic NRXN1 and NRXN1–NRXN2 variants

Patients	Our proband	Zweier et al. (2009)	Harrison et al. (2011)—sibling 1	Harrison et al. (2011)—sibling 2	Duong et al. (2012)	Béna et al. (2013)	Imitola et al. (2014)—sibling 1	Imitola et al. (2014)—sibling 2
NRXN variants	NRXN1: c.2686C>T; p.Arg896Trp NRXN2: c.3176G>A; p. Arg1059Gln	NRXN1: 113 kb del (2:51001003–51113677) NRXN1: p. Ser979Ter	NRXN1: 79 kb del (2:50214717–50293739) NRXN1: 287 kb del (2:51008023–51294599)	NRXN1: 79 kb del (2:50214717–50293739) NRXN1: 287 kb del (2:51008023–51294599)	NRXN1: 451 kb del (2:50812891–51263512) NRXN1: c.2880-1G>A; p. Tyr961ThrfsX31	NRXN1: 181 kb del (2:50963194–51144527) NRXN1: 401 kb del (2:50963194–51364465)	NRXN1: 130 kb del (2:51047025–51176742) NRXN1: 135 kb del (2:51184729–51319450)	NRXN1: 107 kb del (2:51047060–51154995) NRXN1: 174 kb del (2:51154996–51328796)
Age	49 d[a]	18 yr	16 yr	11 yr	33 yr	10 yr	7 yr	7 yr
Gender	F	F	F	F	M	F	M	F
Developmental delay	N/A	Severe	Severe	Severe	Severe	Severe	Severe	Severe
Seizure onset	2 d	None	6 mo	4 mo	7 mo	6.5 yr	5 yr	None
Seizure type	EIEE	None	EIEE	EIEE	IS, TC	AA, TC, HT	AA	None
Age of walking	N/A	2 yr	5 yr	None	3 yr	26 mo	2 yr	2 yr
Speech	N/A	None	20–25 words	None	None	Few words	2–10 words	2–5 words
Breathing abnormality	Irregular breathing with periods of severe apnea	Hyperventilation	Hyperventilation	Breath holding	N/A	Recurrent airway infections	No	Hyperventilation
Stereotypies	N/A	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Constipation	N/A	Yes	Yes	Yes	N/A	Yes	Yes	Yes
Abnormal sleep–wake cycle	N/A	Yes	Yes	Yes	N/A	Yes	Yes	Yes
Brain MRI	Normal	N/A	Normal	N/A	Moderate atrophy and cyst deflecting right hippocampus	Normal	Normal	–
Other	Extreme lethargy and hypertonicity of the extremities	Strabismus, protruding tongue, excessive drooling, broad mouth	GORD, scoliosis, pulmonary stenosis, hypotonia, early-onset, puberty, self-harm behavior	GORD, scoliosis, pulmonary stenosis, hypotonia, early-onset, puberty	Strabismus, perceptive hearing impairment, autism, dysmorphic features	Recurrent FS during her first 2 yr, feeding problems and GORD, autism, no obvious dysmorphic features	Dysphagia, aggressive and self-harm behavior, and ventricular septal defect	Aggressive behavior, strabismus, hypotonia and ataxia

(AA) atypical absence, (EIEE) early infantile epileptic encephalopathy, (F) female, (GORD) gastroesophageal reflux disease, (HT) hypotonic seizures, (IS) infantile spasms, (M) male, (MRI) magnetic resonance imaging, (N/A) not available, (TC) tonic–clonic seizures.
[a]Deceased.

NRXN1 and *NRXN2* such as those in our patient act together to produce a severe phenotype. However, we did not perform functional studies of the effect of the specific variants in our patient. There are no other reported human cases with digenic variants in *NRXN1/NRXN2* to which we can compare our patient. Although the *NRXN2* variant is present in three individuals in gnomAD, overall *NRXN2* is very intolerant for missense variants (constraint missense *z*-score of 5.81; http://exac.broadinstitute.org/). In the current study, we performed WES analysis, which is associated with a high sequencing depth of the protein-coding regions. Because WES targets only the protein-coding regions (1%–2% of the genome), we may have missed variants in regulatory regions or small intragenic CNVs, which might account instead for the phenotype. We did evaluate for larger CNVs with a commercial chromosomal microarray.

CONCLUSION

We present a case with EIEE, strikingly suppressed respiratory effort, and arcuate nucleus hypoplasia identified on postmortem research evaluation in the setting of two variants in *NRXN1* and *NRXN2*, one inherited from the patient's mother who had a family history of SIDS and one from her father who had a history of febrile seizures and family history of epilepsy. Patients with biallelic loss of *NRXN1* in humans and double α-neurexin knockout mice have severe breathing abnormalities, corresponding to our patient's respiratory phenotype. Heterozygous *NRXN1* variants can be disease-associated, and the *NRXN1* and *NRXN2* proteins have a known interaction. Taken together, these findings lead us to hypothesize that our patient's digenic variants in *NRXN1* and *NRXN2* contributed to the combined phenotype of EIEE and arcuate nucleus hypoplasia, which in turn contributed to her respiratory failure and ultimately death. We hypothesize that the neuroanatomical abnormalities identified played a role in brainstem-mediated respiratory dysfunction, through mechanisms similar to those hypothesized for SIDS and also associated with loss of function of neurexin.

METHODS

Neuroimaging Technique

Ex vivo postmortem imaging was performed after fixation of the brain on a 3-Tesla scanner using a 64-channel coil. High-resolution T1- and T2-weighted imaging of the whole brain was performed at 300-micron resolution. Additional 200-micron resolution small field of view imaging of the hippocampi was performed as well. Finally, multishell multi B-value 1 mm isotropic resolution diffusion-weighted imaging was performed.

Genomic Analyses

The microarray was performed by Claritas Genomics using a 4 × 180k Agilent array with a resolution of 5–20 kb for known disease regions and around OMIM genes and with a backbone resolution of 100 kb.

DNA extracted from whole blood underwent capture for exome sequencing using the Agilent SureSelect XTHuman All Exon v4 (Broad Institute). Sequencing of 100-bp paired-end reads was obtained using Illumina HiSeq (Illumina). Coverage was >90% meeting 20× coverage. Our data analysis and variant calling methods have been described previously (Olson et al. 2017). We utilized the BCH Connect Genomics Gateway integrated with the WuXi NextCODE analysis platform (Gudbjartsson et al. 2016) for variant interrogation and analysis. We performed WES for the proband and his parents and evaluated for de novo, homozygous recessive and/or compound heterozygous variants. In addition, we filtered the

WES data for variants in a large list of candidate genes ($n = 512$) and for disease-associated genes. Functional prediction scores were obtained from the dbNSFP database version 3.5 (August 2017; http://varianttools.sourceforge.net/). In total we used five prediction scores (SIFT, PolyPhen-2-HVAR, PolyPhen-2-HDIV, MutationTaster, CADD) and one conservation score (GERP++). We also looked for the functional constraint gene scores (Lek et al. 2016). We classified the pathogenicity of the variants using the ACMG guidelines (Richards et al. 2015). We checked Decipher (https://decipher.sanger.ac.uk) for additional CNVs in *NRXN1/2*.

ADDITIONAL INFORMATION

Ethics Statement
The study was approved by the Boston Children's Hospital Institutional Review Board, with full written consent obtained from the parents, providing consent for the proband.

Author Contributions
All authors were involved in the conception or design of the study, analysis and interpretation of the data, and/or drafting the article or revising it critically for important intellectual content.

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