HHS Public Access

Author manuscript

Neurogenetics. Author manuscript; available in PMC 2016 July 01.

Published in final edited form as:

Neurogenetics. 2016 July ; 17(3): 159–164. doi:10.1007/s10048-016-0479-z.

Mutations in *HIVEP2* are associated with developmental delay, intellectual disability and dysmorphic features

Hallie Steinfeld[1], **Megan T. Cho**[2], **Kyle Retterer**[2], **Rick Person**[2], **G. Bradley Schaefer**[3], **Noelle Danylchuk**[3], **Saleem Malik**[4], **Stephanie Burns Wechsler**[5], **Patricia G. Wheeler**[6], **Koen L.I. van Gassen**[7], **P.A. Terhal**[7], **Virginie J.M. Verhoeven**[8], **Marjon A. van Slegtenhorst**[8], **Kristin G. Monaghan**[2], **Lindsay B. Henderson**[2], and **Wendy K. Chung**[1,9]

[1]Department of Pediatrics, Columbia University Medical Center, New York, NY, USA [2]GeneDx, Gaithersburg, MD, USA [3]Arkansas Children's Hospital, Little Rock, AR, USA [4]Cook Children's Neurology, Ft. Worth, TX, USA [5]Duke University Medical Center, Durham, NC, USA [6]Nemours Children's Hospital, Orlando, FL, USA [7]Department of Genetics, University Medical Center Utrecht, Utrecht 3584, the Netherlands [8]Department of Clinical Genetics, Erasmus Medical Center, Rotterdam, the Netherlands [9]Department of Medicine, Columbia University Medical Center, New York, NY, USA

Abstract

HIVEP2 (human immunodeficiency virus type I enhancer binding protein 2) has been previously associated with intellectual disability and developmental delay in three patients. Here we describe six patients with developmental delay, intellectual disability and dysmorphic features with *de novo* likely gene damaging variants in *HIVEP2* identified by whole exome sequencing (WES). *HIVEP2* encodes a large transcription factor that regulates various neurodevelopmental pathways. Our findings provide further evidence that pathogenic variants in *HIVEP2* lead to intellectual disabilities and developmental delay.

Keywords

HIVEP2; developmental delay; intellectual disability; *de novo*; whole exome sequencing

Introduction

HIVEP2 (human immunodeficiency virus type I enhancer binding protein 2), also referred to as MIBP1, ZAS2, ZNF40B, MBP-2, and Schnurri-2, is a gene that has been associated with intellectual disability and developmental delay [1, 2]. *HIVEP2* encodes a large transcription factor that regulates various neurodevelopmental pathways. To date, three patients with loss-of-function mutations in *HIVEP2* have been published, all with developmental delay,

Correspondence: Wendy K. Chung, Department of Pediatrics, Columbia University Medical Center, 1150 St. Nicholas Avenue, New York, NY 10032, USA, Tel: +1 212 851 5313, Fax: +1 212 851 5306, wkc15@columbia.edu.

Competing Interests Statement

Megan Cho, Kyle Retterer, Rick Person, Kristin Monaghan, and Lindsay Henderson are employees of GeneDx. Wendy Chung is a consultant to BioReference Laboratories.

intellectual disability, hypotonia, and dysmorphic features [1]. In this paper, we present six additional patients with *de novo* variants in *HIVEP2*, identified through WES, with phenotypes similar to those previously published as well as some additional clinical features. Five of the patients have predicted loss-of-function variants while the sixth has a missense variant that is predicted to be damaging. Our cases provide further evidence that pathogenic variants in *HIVEP2* cause neurodevelopmental disorders.

Methods

This study was approved by the Institutional Review Board of Columbia University. Informed consent was obtained from all individual participants included in the study. Additional informed consent was obtained from all individual participants for whom identifying information is included in this article. Genomic DNA was extracted from whole blood from the affected children and their parents. Exome sequencing for patients 1–4 was performed on exon targets isolated by capture using the Agilent Clinical Research Exome kit (Agilent Technologies, Santa Clara, CA). The sequencing methodology and variant interpretation protocol for patients 1–4 has been previously described [3]. The general assertion criteria for variant classification are publicly available on the GeneDx ClinVar submission page (http://www.ncbi.nlm.nih.gov/clinvar/submitters/26957/).

Whole exome sequencing for patient 5 was performed using the SureSelect XT Human All Exon V5 kit (Agilent) at a mean target depth of 100×. The target is defined as all coding exons of UCSC and Ensembl +/− 20bp intron flanks. Reads for patients 5 and 6 were aligned to Hg19 using BWA (BWA-MEM v0.7.5a) and variants were called using the GATK haplotype caller (v2.7-2). Detected variants were annotated, filtered and prioritized using the Bench lab NGS v.3.1.2 platform (Cartagenia, Leuven, Belgium). *De novo* mutation analysis, by filtering all detected variants against parental and population variants were confirmed by Sanger sequencing. For patient 6, the laboratory used the SureSelectXT Clinical Research Exome (Agilent) kit.

Results

WES was performed on 3,699 patients with neuro-developmental disorders within the study time period of patients 1–4. For those four patients, WES produced an average of ~9GB of sequence per sample. Mean coverage of captured regions was ~135X per sample, with >95% covered with at least 10x coverage, an average of >94% of base call quality of Q30 or greater, and an overall average mean quality score of >Q36. Filtering of common SNPs, manual curation, evaluation of predicted effects of rare variants and known function of the genes and associated human conditions, and examination of overlapping phenotypes of individuals with *de novo* variants in the same gene identified variants in *HIVEP2* as likely gene damaging in four probands in the series of 3,699 patients (0.1%). Two additional patients with *de novo* likely gene damaging variants in *HIVEP2* were identified through GeneMatcher[4].

Six unrelated patients ranging in age from 2 to 14 years of age were found to have novel, heterozygous *de novo* variants in *HIVEP2*: two nonsense, three frameshift, and one missense

variant (Figure 1). Maternity and paternity were confirmed as part of quality control in the WES analysis, and alle *HIVEP2* variants were confirmed by Sanger sequencing in the proband and both parents and were *de novo* and absent in both parents. None of the variants is present in ExAC[5] and RVIS score for this gene is −2.28 (1.36%), indicating that the gene is intolerant to variation[6]. The missense variant is a non-conservative amino acid substitution (p.Asp397Tyr) and is located at a conserved position. In silico tools SIFT, Provean, Mutation Taster, and CADD all predict it to be pathogenic[7–9]. It was classified as a likely pathogenic variant using the Richards et al. criteria[10].

All six patients have developmental delay, intellectual disabilities, and mild dysmorphic features (Figure 2). Four of the children have hypotonia, and one has hypertonia. Gross motor milestones were delayed. Two of the children are nonverbal while another has only three words. One child can speak in full sentences but also imitates and is difficult to understand, and the six year old child speaks in only 3–4 word sentences. IQ was not consistently available but ranged from 50–75. The clinical course was largely one of severe delay in development but some gains in developmental milestones. Two of the children have seizures. Behavioral issues are common and include autism, hyperactivity, anxiety, and oppositional behaviors. MRI of the brain did not generally demonstrate structural anomalies although there was mild volume loss in patient and another patient had incomplete myelination at 4 years old. Notably, three of the children are microcephalic and another child has a head circumference < 10%. Medical issues are not common, and gastrointestinal issues of constipation and gastroesophageal reflux disease are reported. Ophthalmologic issues were reported in three patients and included strabismus and amblyopia.

Five individuals with chromosomal deletions of 6q24.2 ranging in size from 6 to 20 Mb and including *HIVEP2* have been described [11]. All five individuals have neurodevelopmental delay, and some have additional features including short stature and/or craniosynostosis, some features of which may be due to deletion of neighboring genes.

Discussion

We describe six patients with common clinical features of neurodevelopmental disorders including developmental delay/intellectual disabilities and dysmorphic features with heterozygous *de novo* likely gene damaging novel variants in *HIVEP2*, identified through WES. Our series include one de novo missense variant, p.D397Y, which is predicted to be damaging but has not been functionally assessed. The phenotype of patient 4 who carries the p.D397Y variant overlaps with the other patients in our series but is nonspecific. Most of the children are nonverbal or minimally verbal. Additional less consistent features in our series include microcephaly without associated brain malformations, seizures and a range of behavioral problems commonly observed in individuals with intellectual disabilities including autism, attention deficit, oppositional defiance, and anxiety. Our series is consistent with and expands the phenotype previously reported by Srivastava et al describing three patients with loss of function *de novo* variants in *HIVEP2*[1]. Common features across all nine patients include hypotonia, developmental delay, intellectual disability, and dysmorphic features (Table 1) [1] [2].

HIVEP2 is hypothesized to cause disease through its involvement in the somatostatin receptor pathway, which has been previously associated with intellectual disability[2]. Our six additional patients provide further evidence that loss-of-function mutations in *HIVEP2* cause neurodevelopmental disease.

HIVEP2 is located on 6q23–q24[12]. It is expressed in the brain and encodes a 2446 amino acid protein that is a member of the ZAS protein family, along with HIVEP1 and HIVEP3. ZAS proteins all contain two zinc finger pairs, a serine/threonine-rich sequence and an acidic region, and encode large transcription factors involved in growth and development [13]. With specificity provided by the zinc fingers, HIVEP2 binds to the DNA sequence 5′-GGGACTTTCC-3′ in enhancer elements of viral and gene promoters.

The predominance of neurodevelopmental features observed in our six patients, along with the three previously published patients, may be explained by HIVEP2's role in brain development. HIVEP2 has been shown to enhance expression of somatostatin receptor type II (SSRT-2) in the brain. HIVEP2 binds with the initiator-binding protein TCF4 to a TC-rich site in the SSRT-2 promoter and activates transcription. SSRT-2 and HIVEP2 are co-expressed in the frontal cortex and hippocampus [14]. HIVEP2 is also known to repress transcription of key developmental genes, including c-myc, a transcription factor that regulates cell growth, differentiation and apoptosis [15]as well as genes in the NF-κB pathway, which play a role in neuronal development, function, and survival [16, 17].

Furthermore, mouse models demonstrate the gene's role in neurologic function, primarily in the hippocampus. *Hivep2*-knockout mice show decreased maturation of neurons and expression of dopamine receptors in the dentate gyrus region of the hippocampus. Phenotypically, *Hivep2*-knockout mice demonstrate behavioral abnormalities including anxiety, hyperactivity, working memory deficits, reduced pre-pulse inhibition, and impaired sociability, features consistent with those observed in our patients. [1, 18]

The six patients in our series support the hypothesis that loss of function variants in *HIVEP2* are a rare cause of neurodevelopmental abnormalities.

Acknowledgments

We gratefully acknowledge the contribution of the patients and their families. This work is supported by a grant from the Simons Foundation.

References

1. Srivastava S, et al. Loss-of-function variants in HIVEP2 are a cause of intellectual disability. Eur J Hum Genet. 2015
2. Rauch A, et al. Range of genetic mutations associated with severe non-syndromic sporadic intellectual disability: an exome sequencing study. Lancet. 2012; 380(9854):1674–82. [PubMed: 23020937]
3. Tanaka AJ, et al. Mutations in SPATA5 Are Associated with Microcephaly, Intellectual Disability, Seizures, and Hearing Loss. Am J Hum Genet. 2015; 97(3):457–64. [PubMed: 26299366]
4. Sobreira N, Schiettecatte F, Valle D, Hamosh A. GeneMatcher: a matching tool for connecting investigators with an interest in the same gene. Hum Mutat. 2015; 36(10):928–30. [PubMed: 26220891]

5. Exome Aggregation Consortium (ExAC). Available from: http://exac.broadinstitute.org
6. Petrovski S, et al. Genic intolerance to functional variation and the interpretation of personal genomes. PLoS Genet. 2013; 9(8):e1003709. [PubMed: 23990802]
7. Choi Y, Chan AP. PROVEAN web server: a tool to predict the functional effect of amino acid substitutions and indels. Bioinformatics. 2015; 31(16):2745–7. [PubMed: 25851949]
8. Kumar P, Henikoff S, Ng PC. Predicting the effects of coding non-synonymous variants on protein function using the SIFT algorithm. Nat Protoc. 2009; 4(7):1073–81. [PubMed: 19561590]
9. Adzhubei IA, et al. A method and server for predicting damaging missense mutations. Nat Methods. 2010; 7(4):248–9. [PubMed: 20354512]
10. Richards S, et al. Standards and guidelines for the interpretation of sequence variants: a joint consensus recommendation of the American College of Medical Genetics and Genomics and the Association for Molecular Pathology. Genet Med. 2015; 17(5):405–24. [PubMed: 25741868]
11. Firth HV, et al. DECIPHER: Database of Chromosomal Imbalance and Phenotype in Humans Using Ensembl Resources. Am J Hum Genet. 2009; 84(4):524–33. [PubMed: 19344873]
12. Sudo T, et al. Mapping of the human gene for the human immunodeficiency virus type 1 enhancer binding protein HIV-EP2 to chromosome 6q23–q24. Genomics. 1992; 12(1):167–70. [PubMed: 1733857]
13. Wu LC. ZAS: C2H2 zinc finger proteins involved in growth and development. Gene Expr. 2002; 10(4):137–52. [PubMed: 12173742]
14. Dorflinger U, et al. Activation of somatostatin receptor II expression by transcription factors MIBP1 and SEF-2 in the murine brain. Mol Cell Biol. 1999; 19(5):3736–47. [PubMed: 10207097]
15. Fukuda S, et al. Characterization of the biological functions of a transcription factor, c-myc intron binding protein 1 (MIBP1). J Biochem. 2002; 131(3):349–57. [PubMed: 11872163]
16. O'Neill LA, Kaltschmidt C. NF-kappa B: a crucial transcription factor for glial and neuronal cell function. Trends Neurosci. 1997; 20(6):252–8. [PubMed: 9185306]
17. Mattson MP. NF-kappaB in the survival and plasticity of neurons. Neurochem Res. 2005; 30(6–7): 883–93. [PubMed: 16187223]
18. Takao K, et al. Deficiency of schnurri-2, an MHC enhancer binding protein, induces mild chronic inflammation in the brain and confers molecular, neuronal, and behavioral phenotypes related to schizophrenia. Neuropsychopharmacology. 2013; 38(8):1409–25. [PubMed: 23389689]

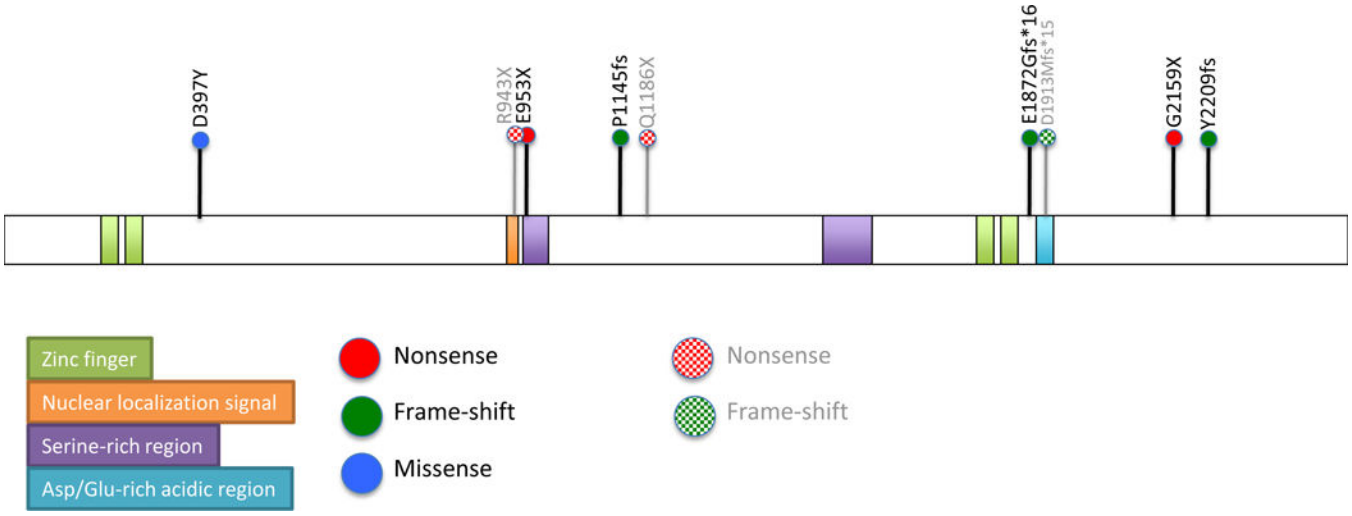
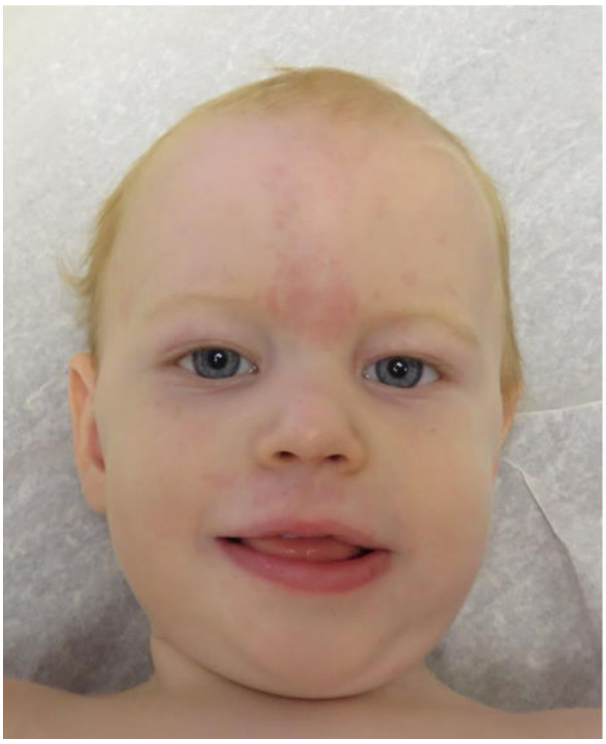


Fig. 1.

Predicted pathogenic variants in *HIVEP2*. Variants in the solid symbols are reported in this manuscript. Previously published cases are included in checkered symbols.



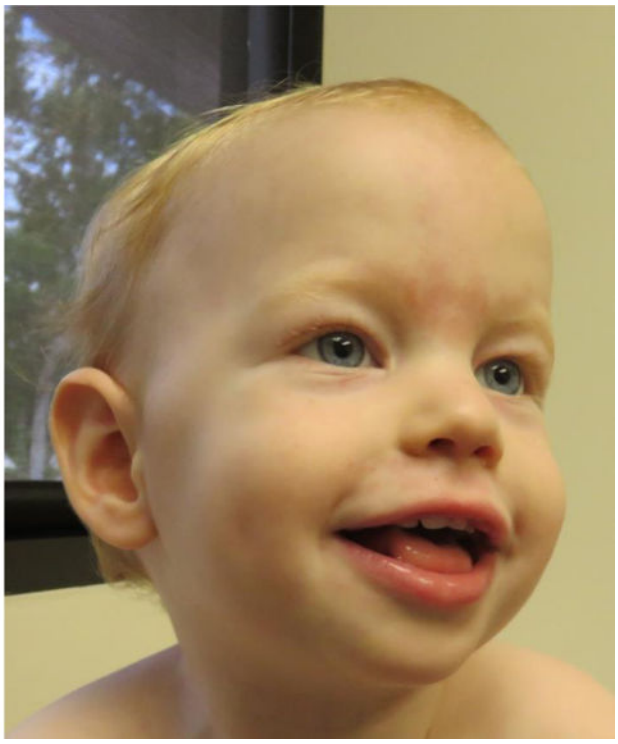


Fig. 2.

Facial features of Patient 4 which include mild dolichocephaly with a tall forehead, mild hypertelorism, and a glabellar nevus flammeus.

Neurogenetics. Author manuscript; available in PMC 2016 July 01.

Table 1

th likely gene damaging variants in *HIVEP2*, including previously published cases.

	Patient 2	**Patient 3**	**Patient 4**	**Patient 5**	**Patient 6**	**Srivastava et al. #1**	**Srivastava et al. #2**	**Srivastava et al. #3**
T K	c.G2857T p.E953X	c.5614dupG p.E1872Gfs*16	c.G1189T p.D397Y	c.6625dup p.Y2209fs	c.3434delC p.P1145fs	c.C2827T p.R943X	c.C3556T p.Q1186X	c.5737delGp.D1 913Mfs*15
	14	10	2	11	6	4	3	21
	Female	Male	Male	Female	Male	Female	Male	Female
e contractions, l fetal movement	None	First trimester vaginal bleeding, decreased fetal movement	Decreased amniotic fluid	None	None	First-trimester vaginal bleeding	None	None
	N	N	N	N	N	N	N	Umbilical hernia, mild pulmonary stenosis
7 g (90%) 34 cm (90%), K	WT= 3062 g (25%) HT=48.26 cm (25–50%), OFC = 32.4 cm (Z=−1.8)	WT = 3856 g (~75%), HT = 53.3 cm (~90%), OFC UNK	WT= 3770 g (50 – 75%), HT & OFC UNK	WT = 3880 g (75–90%), HT & OFC UNK	WT= 4100 g (90%)HT & OFC UNK	WT= 3150 g (25–50 %)	HT= 53cm (75th–90th centile), WT= 3330g (25th–50th centile), OFC= 35cm (Z=−0.4)	HT= 50cm (25th centile), WT= 2750g (10th centile), OFC= 32cm (Z=−2)
3 kg (50 %) cm (75 %), .5 cm (Z=−2.4)	WT= 47.5 kg (25–50%), HT= 149.5 cm (50%) OFC= 51 cm (Z=−2.6)	WT= 31.4 kg (25–50%), HT= 140.9 cm (50–75%) OFC= 48.7 cm (Z=−3.2)	WT= 10.6 kg (<5 %), HT= 80.8 cm (<3%), OFC= 46.7 cm (Z=−1.7	WT = 44 kg (50–75 %), HT = 152 cm (50–75%). OFC at 3yo10mos = 50.8 (Z=0.7)	WT= 24 kg (50–75 %), HT= 120 cm (50 %), OFC= 52cm (Z=0.3)	WT= 17.1 kg (85%), HT= 100 cm (70%), OFC= 48 cm (Z=−1.5)	WT= 11 kg (25–50 %), HT= 80 cm (25 %), OFC= 48 cm (Z=−1.4)	WT= 59kg (25–50%), HT= 164 cm (25 –50 %), OFC= 52.2 cm (Z=−2)
ognathia	Elongated and narrow face	Low anterior hairline, hirsute, prominent eyebrows, synophrys, epicanthal folds, mildly thickened helices & simple antihelices in ears, mild dental crowding of lower jaw	Small hands and feet	Somewhat high nasal bridge, broad mouth, rather flat philtrum, mild bifrontal narrowing, mildly broad halluces, sacral dimple, broad thorax, mild finger webbing	Square face, high/broad forehead, unilateral strabismus, high nasal bridge, columella under alae nasi, small square ears with transverse crease, small square teeth, microretrognathia	Widely set eyes, broad nasal root, slightly upturned nose, high-arched palate	High forehead, medial eyebrow flare, widely set eyes, broad nasal root, small mouth, slightly tapering fingers, flat feet	Upslanting palpebral fissures, mild synophrys, small ears with attached earlobes, prominent nose with high nasal bridge and columella extending below the alae nasi, short philtrum, thin upper lip, overbite, hypertrophic gingiva, tapering fingers, radial deviation of 4th fingers, wide feet with short toes, hirsutism
	Y	Y	Y	Y	Y	Y	Y	Y
	12	28	7	14	9	UNK	13	9

	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Srivastava et al. #1	Srivastava et al. #2	Srivastava et al. #3
	36	With walker, 30–36 months; without walker 4 to 5 years	Not yet	30	22	36	30	36
	N/A	N/A	24 mos	10 mos	18 mos	24 mos	24 mos	7 yo
short sentences; words by 3 yo	No verbal speech	No verbal speech	3 words	Full sentences and imitation; difficult to understand	Sentences with 3–4 words	Language age equivalent of 15mos		Partially slurred articulation
a	Hypotonia	Hypertonia	Hypotonia	Hypotonia	N	Hypotonia	Hypotonia	Hypotonia
	Y	N	N	N	N	Unavailable	Unavailable	Unavailable
e	Y	Y	N/A	Y	Y	Y	Y	Y
	UNK	UNK	N/A	50	50–70	Unavailable	Unavailable	Unavailable
rocessing disorder	N	Sensory integration disorder	N/A		N	Unavailable	Unavailable	Unavailable
	Mild volume loss	Normal structure MRI at age 6 yo; abnormal MR spectroscopy. Normal structure MRI at age 9 yo	Normal	Incomplete myelination at age 4 yo	Normal	Slightly thin corpus callosum	Hypoplasia of the corpus callosum	Mild frontal atrophy
nging, nal/de fiant	Hands in hair, hands up like puppet, wringing of hands	Tic like head jerking at one point. Impulsive, distractible	N	Sensitive to stimuli, requires structure	Hyperactivity, concentration problems, anxiety	Hyperactivity, impulsivity, distractibility	None	Aggression, impulsivity, self-stimulation, hyperactivity
al pain, GERD	Constipation, GERD	GERD, milk protein intolerance as infant	Short stature	N	N	Gastroparesis and projectile vomiting	None	Constipation
	Strabismus	Strabismus (esotropia) s/p eye muscle surgery at age 6 yo	N	N	Amblyopia and strabismus, high hypermetropia (+5/+6 diopters)	N	Hypermetropia	N
	Y	Y	N	N	N	N	N	N
EG	Mostly tonic seizures, history of myoclonic and complex partial seizures EEG: multiple tonic seizures, focal seizures, spike and slow wave- bifrontal	Febrile (one) and afebrile (one)	EEG abnormality: left occipital sharp wave and spike/wave discharges that increase as he progresses into sleep.	N/A	N/A	Normal EEG	Normal EEG	Normal EEG
al	Daily, Lennox Gastaut Syndrome	Rare (2 in 10 years)	N/A	N/A	N/A	N/A	N/A	N/A

	Patient 2	Patient 3	Patient 4	Patient 5	Patient 6	Srivastava et al. #1	Srivastava et al. #2	Srivastava et al. #3
ephalopathy, exercise ce/easily fatigued	Ataxia, spasticity, cerebral palsy, quadriplegia, muscle weakness, dystonia	Spasticity, muscle weakness, tremors, progressive Parkinsonism, right hemiparesis, tongue fasciculations	N	Dyspraxia	N	Frequent head tilt and leftward eye movements with preserved ability to fix/ follow objects, subtle left facial weakness, wide based gait	Clumsy gait	Ataxia, dystonia
ure instability	Iron deficiency anemia, nephrocalcinosis on renal US, hip dysplasia, irregular menstrual periods, syncope, dyspnea	Frequent tonsillitis, improved after T & A at age 9	Reactive airway disease; AFOs primarily for ankles rolling due to hypotonia	Hypermobility of fingers, increased inversion of feet and decreased eversion	Bronchial hyperreactivity, breath holding spells	Asymmetric cry, unilateral hip dysplasia, dysphagia	Significant salivation	Hypertrophic gingiva with frequent bleeding, high TSH levels

ntal circumference, UNK=unknown, GERD=gastroesophageal reflux disease, s/p = status post, T & A = tonsillectomy & adenoidectomy, mos = months old, yo