Bi-allelic variants in *INTS11* are associated with a complex neurological disorder

Authors

Burak Tepe, Erica L. Macke, Marcello Niceta, ..., Marco Tartaglia, Eric W. Klee, Hugo J. Bellen

Correspondence

hbellen@bcm.edu

Here we describe fifteen individuals who carry damaging bi-allelic variants in *INTS11* and who present with neurological symptoms. Modeling experiments in *Drosophila* identified two strong and five mild LoF alleles of *INTS11*. Our results provide compelling evidence that the integrity of the Integrator RNA endonuclease is critical for brain development.

Tepe et al., 2023, The American Journal of Human Genetics *110*, 774–789
May 4, 2023
https://doi.org/10.1016/j.ajhg.2023.03.012

CellPress

ARTICLE

Bi-allelic variants in *INTS11* are associated with a complex neurological disorder

Burak Tepe,[1,2,27] Erica L. Macke,[3,27] Marcello Niceta,[4,27] Monika Weisz Hubshman,[1] Oguz Kanca,[1,2] Laura Schultz-Rogers,[3] Yuri A. Zarate,[5] G. Bradley Schaefer,[6] Jorge Luis Granadillo De Luque,[7] Daniel J. Wegner,[8] Benjamin Cogne,[9] Brigitte Gilbert-Dussardier,[10] Xavier Le Guillou,[10] Eric J. Wagner,[11] Lynn S. Pais,[12,13] Jennifer E. Neil,[12] Ganeshwaran H. Mochida,[12,14] Christopher A. Walsh,[12] Nurit Magal,[15] Valerie Drasinover,[15] Mordechai Shohat,[16,17] Tanya Schwab,[3] Chris Schmitz,[3] Karl Clark,[3] Anthony Fine,[18] Brendan Lanpher,[19] Ralitza Gavrilova,[19] Pierre Blanc,[20] Lydie Burglen,[20] Alexandra Afenjar,[21] Dora Steel,[22,23] Manju A. Kurian,[22,23] Prab Prabhakar,[23] Sophie Gößwein,[24] Nataliya Di Donato,[24] Enrico S. Bertini,[25] Undiagnosed Diseases Network, Michael F. Wangler,[1,2] Shinya Yamamoto,[1,2,26] Marco Tartaglia,[4] Eric W. Klee,[3,19] and Hugo J. Bellen[1,2,26,*]

Summary

The Integrator complex is a multi-subunit protein complex that regulates the processing of nascent RNAs transcribed by RNA polymerase II (RNAPII), including small nuclear RNAs, enhancer RNAs, telomeric RNAs, viral RNAs, and protein-coding mRNAs. *Integrator subunit 11* (*INTS11*) is the catalytic subunit that cleaves nascent RNAs, but, to date, mutations in this subunit have not been linked to human disease. Here, we describe 15 individuals from 10 unrelated families with bi-allelic variants in *INTS11* who present with global developmental and language delay, intellectual disability, impaired motor development, and brain atrophy. Consistent with human observations, we find that the fly ortholog of *INTS11*, *dIntS11*, is essential and expressed in the central nervous systems in a subset of neurons and most glia in larval and adult stages. Using *Drosophila* as a model, we investigated the effect of seven variants. We found that two (p.Arg17Leu and p.His414Tyr) fail to rescue the lethality of null mutants, indicating that they are strong loss-of-function variants. Furthermore, we found that five variants (p.Gly55Ser, p.Leu138Phe, p.Lys396Glu, p.Val517Met, and p.Ile553Glu) rescue lethality but cause a shortened lifespan and bang sensitivity and affect locomotor activity, indicating that they are partial loss-of-function variants. Altogether, our results provide compelling evidence that integrity of the Integrator RNA endonuclease is critical for brain development.

Introduction

The Integrator complex is a multi-subunit protein complex consisting of 15 subunits (IntS1-15)[1] that regulates the termination of RNA polymerase II (RNAPII)[2] (Figure S1). Initially, the Integrator was discovered as a complex performing 3′ end processing of small nuclear RNAs, critical components of the splicing machinery.[3,4] Later, the repertoire of non-coding RNAs processed by Integrator was expanded to include some enhancer RNAs,[5] telomeric RNAs,[6] and viral RNAs.[7,8] Most recently, the Integrator has been found to be a broad driver of promoter-proximal termination at all RNAPII loci, including protein-coding genes.[9–15] The RNA-processing activity of the Integrator complex is carried out by subunit 11 (INTS11), also known as Cleavage and Polyadenylation Specific Factor 3-Like

[1]Department of Molecular and Human Genetics, Baylor College of Medicine, Houston, TX 77030, USA; [2]Jan and Dan Duncan Neurological Research Institute at Texas Children's Hospital, Houston, TX 77030, USA; [3]Center for Individualized Medicine, Mayo Clinic, Rochester, MN 55905, USA; [4]Molecular Genetics and Functional Genomics, Ospedale Pediatrico Bambino Gesù, IRCCS, Rome, Italy; [5]Division of Genetics and Metabolism, University of Kentucky, Lexington, KY, USA; [6]Section of Genetics and Metabolism, University of Arkansas for Medical Sciences, Little Rock, AR, USA; [7]Division of Genetics and Genomic Medicine, Department of Pediatrics, Washington University School of Medicine, St. Louis, MO, USA; [8]Edward Mallinckrodt Department of Pediatrics, Washington University in St. Louis School of Medicine and St. Louis Children's Hospital, St. Louis, Missouri, USA; [9]Laboratory of Molecular Genetics, CHU de Nantes, Nantes, France; [10]Department of Medical Genetics, CHU de Poitiers, Poitiers, France; [11]Department of Biochemistry and Biophysics, Center for RNA Biology, University of Rochester School of Medicine, Rochester, NY 14642, USA; [12]Division of Genetics and Genomics, and Howard Hughes Medical Institute, Boston Children's Hospital, and Departments of Pediatrics and Neurology, Harvard Medical School, Boston, MA, USA; [13]Program in Medical and Population Genetics, Broad Institute of MIT and Harvard, Cambridge, MA, USA; [14]Department of Neurology, Massachusetts General Hospital, Boston, MA, USA; [15]The Raphael Recanati Genetic Institute, Rabin Medical Center, Petach Tikva, Israel; [16]Cancer Research Center, Chaim Sheba Medical Center, Ramat Gan, Israel; [17]Medical Genetics Institute of Maccabi HMO, Rechovot, Israel; [18]Department of Neurology, Mayo Clinic, Rochester, MN 55905, USA; [19]Department of Clinical Genomics, Mayo Clinic, Rochester, MN 55905, USA; [20]APHP, Département de génétique, Sorbonne Université, GRC n°19, ConCer-LD, Centre de Référence déficiences intellectuelles de causes rares, Hôpital Armand Trousseau, 75012 Paris, France; [21]APHP. SU, Centre de Référence Malformations et maladies congénitales du cervelet, département de génétique et embryologie médicale, Hôpital Trousseau, 75012 Paris, France; [22]Developmental Neurosciences, Zayed Centre for Research into Rare Disease in Children, UCL Great Ormond Street Institute of Child Health, London, UK; [23]Department of Neurology, Great Ormond Street Hospital for Children, London, UK; [24]Institute for Clinical Genetics, University Hospital Carl Gustav Carus at the Technische Universität Dresden, Fetscherstrasse 74, 01307 Dresden, Germany; [25]Unit of Neuromuscular and Neurodegenerative Disorders, Ospedale Pediatrico Bambino Gesù, IRCCS, Rome, Italy; [26]Department of Neuroscience, Baylor College of Medicine, Houston, TX 77030, USA
[27]These authors contributed equally
*Correspondence: hbellen@bcm.edu
https://doi.org/10.1016/j.ajhg.2023.03.012.

(CPSF3L), which contains a metallo-β-lactamase and β-CASP domain that, together, coordinate two zinc ions within the active site to promote its RNA endonuclease activity.[16] INTS11 also forms a heterotrimer with INTS4 and INTS9 to create what has been termed the "Integrator Cleavage Module."[17–21] All three subunits within the Integrator Cleavage Module were shown to be important for Integrator complex function.[22]

The Integrator complex subunits are conserved throughout metazoan lineages. There is only one predicted *INTS11* ortholog in *Drosophila*, also called *IntS11*, with a DIOPT[23] score of 13/16. Previous studies of fly *IntS11* were limited to *in vitro* systems and mutant lines have not been generated to allow organismal investigations.[3,24,25] However, genome-wide RNAi studies in *Drosophila* have shown that reducing the level of *IntS11* in neuroblasts causes a mild reduction in the number of daughter cells derived from neuroblasts in the larval brain[26] whereas a reduction of the expression in cardiac tissue causes a reduced lifespan.[27]

To date, only three Integrator complex subunits have been linked to human disease. Genetic variants in *Integrator Complex Subunit 1* (*INTS1* [MIM: 611345]) and *Integrator Complex Subunit 8* (*INTS8* [MIM: 611351]) have been associated with rare autosomal-recessive human neurodevelopmental syndromes presenting with severe developmental delay (DD), intellectual disability (ID), lack of speech development, motor impairment, seizures, and similar dysmorphic features of the face and limbs[28,29] (MIM: 618571 and 618572, respectively). Recently, *Integrator Complex Subunit 13* (*INTS13* [MIM: 615079]) variants have been associated with an autosomal-recessive ciliopathy.[30] These studies suggest that Integrator function is critical during human development and that the brain may be particularly sensitive to disruptions in Integrator activity.

Here, we present molecular and clinical data from 15 individuals with bi-allelic variants in the *INTS11* gene. These individuals present a neurodevelopmental phenotype due to defective neurogenesis and/or neurodegenerative processes. We generated null alleles of *IntS11* in flies and show that *IntS11* is an essential gene expressed in a subset of larval and adult CNS neurons and glia. We tested seven of the human *INTS11* variants using orthologous modeling in *Drosophila*. We found that two behaved as strong loss of function (LoF) by failing to rescue lethality, while the others lead to viable flies that exhibit defects in several neurological assays. Our findings indicate that variants in human *INTS11* result in a novel complex neurological disorder.

Material and methods

Identification of *INTS11* variants and proband phenotyping

Variants in *INST11* were identified by whole-exome or -genome sequencing performed on whole-blood DNA from probands identified through clinical diagnostic practice or Institutional Review Board-approved research studies. Variants were prioritized based on allele frequency, inheritance, and gene-phenotype associations. Within this cohort no additional explanatory variants were identified for any individuals. Affected individuals were identified through professional communication, connections through GeneMatcher,[31] and by searching the Undiagnosed Diseases Network (UDN) and the Deciphering Developmental Disorders (DDD) Research Study.[32] Variants were reported according to standardized nomenclature defined by the reference human genome GRCh38 (hg38). All variants are reported on *INTS11* transcript GenBank: NM_001256456.1. Patient consent for participation, phenotyping, and sample collection was obtained through the referring clinical teams. Referring clinicians were requested to complete a comprehensive questionnaire that included sections related to neurodevelopmental screening, behavior, dysmorphology, muscular, cardiac, and other systemic phenotypic features.

To assess allele frequencies in control population, we utilized gnomAD. We did not find any individual in the database carrying the variants p.Gly18Ser, p.Arg23Leu, p.Phe45Ser, p.Leu144Phe, p.Lys402Glu, p.Pro413Ser, p.His420Tyr, p.Val557Aspfs*14, or p.Val557Glu. However, there are alternative variants in gnomAD at some of these residues but none are present as homozygotes: p.Gly18Gly (4.02E−06), p.Arg23Ter (1.78E−05), p.Lys402Arg (4.01E−06), p.Pro413Leu (7.13E−06), p.Arg527Cys (3.75E−05), and p.Val557Leu (9.91E−06). Some variants are present in the control population, but again none are present as homozygotes: p.Gly61Ser (7.96E−06), p.Arg223Trp (8.00E−06), p.Glu224Gly (4.00E−06), p.Arg225Gln (1.42E−05), p.Ala319fs*2 (8.17E−05), p.Val521Met (2.13E−05), p.Arg527fs*44 (1.93E−05), and p.Tyr584Cys (5.26E−06). For some of these residues, there are also alternative variants, none present as homozygotes: p.Gly61Gly (7.08E−6), p.Gly61Asp (3.98E−6), p.Arg223Arg (3.19E−5), p.Arg223Gln (4.00E−6), p.Glu224Glu (4.00E−6), p.Glu224Lys (4.00E−6), p.Ala319Ala (4.80E−5), p.Val521Val (6.38E−6), and p.Arg527Cys (3.75E−5). Allele frequencies are reported in Figure S5.

Fly strains and genetics

All fly strains used in this study were generated in-house or obtained from the Bloomington Drosophila Stock Center (BDSC) or Vienna Drosophila Resource Center (VDRC) and cultured at room temperature unless otherwise noted. The *IntS11^{KozakGal4}* allele was generated using the CRIMIC (CRISPR-Mediated Integration Cassette) technique as described.[33] We determined mutant phenotype of *dIntS11* by crossing *IntS11^{KozakGal4}* allele to a genomic deficiency *(Df) (w[1118]; Df(3R)Exel9025/TM6B, Tb[1])* (BDSC #7995). The recessive lethality associated with the *IntS11^{KozakGal4}* allele was rescued with an 120 kb P[acman] duplication *(Dp)(w[1118];Dp (3;2)GV-CH321-23I13,PBac{y[+mDint2]w[+mC]=GV-CH321-23I13} VK00037/CyO)* (BDRC #90212).[34] We determined the cell-type specific expression pattern of *IntS11* by crossing *IntS11^{KozakGal4}* to *UAS-mCherry.NLS (w[*]; P{w[+mC]=UAS-mCherry.NLS}3)* (BDSC #38424).

The *IntS11* RNAi (RNA interference) lines (*P{GD9692}v33450* and *P{KK100724}VIE-260B*) were obtained from VDRC. *da-Gal4 (w[*]; P{w[+mW.hs]=GAL4-da.G32}UH1, Sb[1]/TM6B, Tb[1])* (BDSC #55851), *Act-GAL4/CyO (y[1] w[*]; P{w[+mC]=Act5C-GAL4}25FO1/CyO, y[+])* (BDSC #4414), *elav-Gal4 (P{w[+mC]=GAL4-elav.L}2/CyO)* (BDSC #8765) and *repo-GAL4 (w[1118]; P{w[+m*]=GAL4}repo/TM3, Sb[1])* (BDSC #7415) were obtained from Bloomington Drosophila Stock Center (BDSC).

Cloning and transgenesis

All transgenic constructs were generated by Gateway (Thermo Fisher Scientific) cloning into the pGW-attB-3xHA plasmid.[35] The human *INTS11* cDNA clone was obtained from BCM human cDNA collection (GenBank: NM_017871.6 transcript). Fly *IntS11* cDNA was generated from total cDNA prepared from adult heads from *y[*]w[*]* animals. Flanking Gateway attB sites were added to the primer used to amplify the *IntS11* coding sequence by PCR and then shuttled to the pDONR223 by BP clonase II (Thermo Fisher Scientific). Variants were generated by Q5 site-directed mutagenesis (NEB), fully sequenced (Sanger), and finally cloned into pGW-attB-3xHA plasmid via LR clonase II (Thermo Fisher Scientific). All expression constructs were inserted into the VK37 (*PBac{y[+]-attP}VK00037*) docking site by φC31-mediated transgenesis.[36]

Fly modeling studies were initiated with families 1–6 and 10. Due to technical problems with generating reagents for the missense variants found in family 1 (p.Phe45Ser) and family 5 (p.Glu224Gly), fly modeling was performed only for the missense variants found in families 2, 3, 4, 6, and 10, a total of 7 variants: p.Arg23Leu, p.Gly61Ser, p.Leu144Phe, p.Lys402-Glu, p.His420Tyr, p.Val521Met, and p.Val557Glu. Families 7–9 were recruited later into the study and these variants were not modeled.

Drosophila behavioral assays

For the bang sensitivity assay,[37] flies were not anesthetized with CO_2 for 24 h before being tested. At the time of testing, flies were transferred to a clean vial without food and vortexed for 15 s at maximum speed. The time to recover to freely moving status (without abnormal falling or flipping) was measured for each fly. The climbing assay[38] was performed similarly. Flies were tapped to the bottom of the vial three times and examined for a negative geotaxis (climbing) response to reach the 2.5 cm and 5 cm mark on the vial. Flies were given a maximum of 60 s to pass the mark. Climbing speed is calculated by dividing the distance (2.5 cm or 5 cm) by the time point the fly passes the mark, and average speeds are calculated by taking the mean of the two values. Flies that did not pass the mark were considered to have a climbing speed of zero.

For survival rate, the surviving adult flies were counted within 1–3 days of eclosion, and expected numbers were calculated based on Mendelian ratios. For lifespan, 1- to 3-day-old eclosed flies were separated and maintained at 25°C, and survival was determined every 2–3 days.

Immunohistochemistry

L3 larval CNS and adult brains were dissected and fixed in 4% paraformaldehyde (PFA) (diluted with 1×PBS from 16% paraformaldehyde solution, EM grade; Electron microscopy sciences Cat:15710) at room temperature for 20 min and washed in PBS containing 0.3% Triton X-100. The primary antibodies were used at the following dilutions: anti-Elav (Embryonic lethal abnormal vision) rat monoclonal: 7E8A10(DSHB), 1:200; anti-Repo (mouse monoclonal: 8D12(DSHB), 1:50 and incubated at 4°C overnight. After washing (4 times), samples were incubated with the secondary antibody conjugated to Alexa 647 (Jackson ImmunoResearch) diluted 1:500 in PBS containing 0.3% Triton X-100 and incubated at room temperature for 2 h. After washing (4 times), samples were mounted in RapiClear (SunJin Lab Co.) and imaged with confocal microscopy.

Results

Individuals with variants in *INTS11* exhibit neurological phenotypes

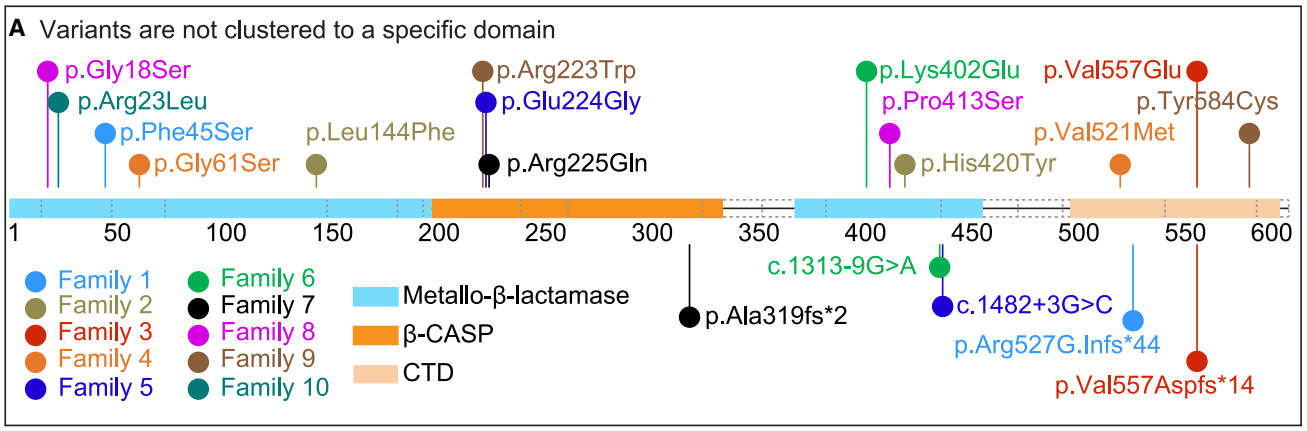
A cohort of 15 individuals from 10 families presented with a neurodevelopmental disorder and harbored either homozygous or compound heterozygous variants in *INTS11* based on exome or whole-genome sequencing (ES and WS, respectively). In total, 19 unique variants were identified: 14 are missense, 2 are intronic variants predicted to impact splicing, 2 are frameshifts resulting in a premature stop codon, and 1 is a nonsense variant (Table 1). The variants span the entire gene and are not clustered (Figure 1A). All variants have Combined Annotation Dependent Depletion (CADD) score[39–41] of higher than 20, and most are predicted to be damaging based on Sorting Intolerant From Tolerant (SIFT) and Polymorphism Phenotyping (PolyPhen)[42] (Figure 1B).

Subjects with bi-allelic variants in *INTS11* coding sequence showed a consistent neurological phenotype. Phenotypic findings are summarized in Table 1, and detailed clinical information for individual families is included in the supplemental information. The cohort consists of 13 females and 2 males, ranging in age from 2 to 18 years. The cohort also includes 5 subjects who died at the ages of 6, 13, and 18 years due to worsening of their clinical conditions. Global DD, ID, motor and speech impairment (delayed or absent), and brain imaging abnormalities were documented in all individuals for whom data were available (14/14). Specifically, MRI brain changes revealed progressive cortical atrophy, cortical gyral simplification, and delayed myelination affecting cerebrum and cerebellum. Delayed gross motor development (12/15) and generalized hypotonia (11/14) were also commonly observed. EEG abnormalities were documented in 6 individuals (6/15), some of whom presented with epilepsy and seizures, which were refractory to antiepileptic drugs (AED) in subject 1 and subject 2. Optic atrophy was documented in 5 subjects (5/14) and visual impairment in 10 (10/14). No hearing loss was documented in the individuals of the cohort. Prenatal and postnatal growth parameters were found to be reduced in several cases (7/11) and certain craniofacial dysmorphisms, including microcephaly, abnormality of cranium shape (i.e., triangular shape and dolichocephaly), hypertelorism, epicanthic fold, narrow chin, flat nasal bridge, cleft of the lip and palate, macroglossia, and retrognathia were also recognized in the affected individuals (11/15). Nonspecific skeletal defects including premature closure of fontanelles, shortened long bones, vertebral flattening, scoliosis, and joint hyperlaxity were documented in some subjects (7/13). Internal organ malformations including heart septal defect and genitourinary anomalies (chronic glomerulopathy) were sporadically identified (Tables 1 and S1).

To gather information about human *INTS11* and its orthologous genes in genetic model organisms, we performed an *in silico* search using MARRVEL (Model organism Aggregated Resources for Rare Variant ExpLoration).[44]

Table 1. Variants in *INTS11* are associated with shared clinical features

	Family 1		Family 2		Family 3	Family 4	Family 5	Family 6	Family 7	Family 8		Family 9	Family 10		
	Subject 1	**Subject 2**	**Subject 3**	**Subject 4**	**Subject 5**	**Subject 6**	**Subject 7**	**Subject 8**	**Subject 9**	**Subject 10**	**Subject 11**	**Subject 12**	**Subject 13**	**Subject 14**	**Subject 15**
Sex	female	female	female	female	female	female	female	female	male	male	female	female	female	female	female
Age	18 years (deceased)	6 years (deceased)	5 years	5 years	8 years	6 years	3 years	16 years	2 years	18 years	17 years	10 years	deceased	13years (deceased)	deceased
Paternal variant	p.Phe45Ser	p.Phe45Ser	p.His420Tyr	p.His420Tyr	p.Val557 Aspfs*14	p.Gly61Ser	p.Glu224Gly	p.Lys402Glu	p.Arg225Gln	p.Pro413Ser	p.Pro413Ser	p.Arg223Trp	p.Arg23Leu	p.Arg23Leu	p.Arg23Leu
Maternal variant	p.Arg527fs*44	p.Arg527fs*44	p.Leu144Phe	p.Leu144Phe	p.Val557Glu	p.Val521Met	c.1482+3G>C	c.1313-9G>A	p.Ala319fs	p.Gly18Ser	p.Gly18Ser	p.Tyr584Cys	p.Arg23Leu	p.Arg23Leu	p.Arg23Leu
Global developmental delay	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Intellectual disability	+++	+++	+++	+++	+	+	+++	+++	+++	+++	+++	+++	+++	+++	+++
Language delay	+++	+++	+++	+++	+	+	+++	++	+++	++	++	++	+++	+++	+++
Motor development and disorders	+++	+++	+	+	+	++	+++	++	+++	+	+	++	+++	+++	+++
Brain MRI	progressive cerebral atrophy, gyral semplification, pontocerebellar atrophy	progressive cerebral atrophy, gyral semplification, pontocerebellar atrophy	cerebellar hypoplasia, abnormal appearance of the posterior fossa	N/A	mild to moderate cerebellar atrophy	subtle peripheral white matter hyperintensity around the occipital horns and to a lesser extent in the frontal regions	simplified gyral pattern with agenesis of the corpus callosum and enlarged ventricles	cerebellar progressive hypoplasia	progressive supratentorial and infratentorial atrophy, leukoencephalopathy	(at age 2 years) small cerebellum; delayed myelination	(at age 9 years) progressive cerebellar atrophy; milder pontine volume loss; diffuse white matter signal changes	cerebellar atrophy	mild brain atrophy	mildly dilated 4th ventricle and suspected mild atrophy of the cerebellum	periventricular signal hyperintensity and was interpreted as suspected delayed myelinization
Hypotonia	yes	yes	yes	yes	N/A	N/A	–	–	yes	yes	yes	–	yes	yes	yes
Seizures	yes	yes	–	–	–	–	yes	–	yes	–	one	–	–	–	–
Microcephaly	yes	yes	–	–	–	–	yes	yes	yes	yes	–	yes	yes	yes	yes
Optic findings	optic atrophy	optic atrophy	strabismus, myopia	myopia	mild optic atrophy	–	N/A	–	optic atrophy	retinal dystrophy	retinal dystrophy; also hypermetropia	astigmatism	–	–	optic atrophy
Prenatal findings	IUGR	IUGR	monozygotic twin	monozygotic twin	–	–	unknown	IUGR	HELLP syndrome	gestational diabetes, mild IUGR	gestational diabetes, premature (33 w)	IUGR	–	microcephaly	–
Facial dysmorphisms	–	–	hypertelorism, triangular face	hypertelorism, triangular face	–	–	fontanelles closed at birth, low set ears	narrow palate, retrognathism, prominent incisors, coarse facies	prominent glabella, receding forehead	–	–	short forehead, epicanthic fold, bifid uvula	coarse face, broad nose and flat nasal bridge, thin upper lip, and wide mouth with macroglossia	coarse facial features, broad nose, large mouth	coarse face, broad nose and flat nasal bridge,



B Most variants are predicted to be damaging

Transcript variant	Protein variant	SIFT	PolyPhen	CADD
c.52G>A	p.Gly18Ser	deleterious(0)	probably_damaging(1)	27.5
c.68G>T	p.Arg23Leu	deleterious(0)	probably_damaging(0.997)	32
c.134T>C	p.Phe45Ser	deleterious(0)	benign(0.139)	23
c.181G>A	p.Gly61Ser	tolerated(0.1)	possibly_damaging(0.606)	23.4
c.430C>T	p.Leu144Phe	deleterious(0.01)	benign(0.248)	23.2
c.667C>T	p.Arg223Trp	deleterious(0)	probably_damaging(0.999)	28
c.671A>G	p.Glu224Gly	deleterious(0)	probably_damaging(1)	31
c.674G>A	p.Arg225Gln	deleterious(0.01)	probably_damaging(0.992)	29.3
c.954dup	p.Ala319fs*2	-	-	26.9
c.1204A>G	p.Lys402Glu	deleterious(0.01)	possibly_damaging(0.559)	26.6
c.1237C>T	p.Pro413Ser	deleterious(0.01)	probably_damaging(0.99)	25.6
c.1258C>T	p.His420Tyr	deleterious(0)	probably_damaging(1)	26.5
c.1313-9G>A		-	-	20.4
c.1482+3G>C		-	-	20.9
c.1561G>A	p.Val521Met	deleterious(0.01)	possibly_damaging(0.511)	25
c.1578_1579delAC	p.Arg527fs*44	-	-	33
c.1670_1671delTG	p.Val557Aspfs*14	-	-	-
c.1670T>A	p.Val557Glu	deleterious_low_confidence(0)	benign(0.192)	23
c.1751A>G	p.Tyr584Cys	tolerated_low_confidence(0.11)	possibly_damaging(0.846)	24.9

C *INTS11* associated symptoms are predicted to show recessive inheritance.

Probability of LoF intolarance (pLI)			Missense constraint			DOMINO	
Expected	Observed	pLI score	Expected	Observed	z score	Score	Inheritance prediction
36.3	26	0	387.1	357	0.54	0.067	Very likely recessive

Figure 1. Most variants in *INTS11* are predicted to be deleterious

(A) Domain structure of human INTS11 protein. INTS11 is 606 amino acid (aa) long and contains three domains: metallo-β-lactamase (light blue), β-CASP (orange), and C-terminal domain (CTD) (tan). Variants identified in this study are indicated above/below the protein as dots and are not clustered in a specific domain.

(B) Table summarizing the nature of the variants and their predicted consequence on protein function. Coding changes are described using the GenBank: NM_001256456.1 transcript. Variants modeled in this study are highlighted in yellow. Variants predicted to be damaging based on SIFT or PolyPhen are highlighted in red (SIFT, Sorting Intolerant From Tolerant; PolyPhen, Polymorphism Phenotyping; CADD, Combined Annotation Dependent Depletion).

(C) *INTS11* is predicted to cause a recessive disease. *INTS11* is not constrained based on the presence of LoF variants in gnomAD,[43] resulting in a low probability-of-LoF-intolerance (pLI) score and missense constraint *Z* score. Based on gnomAD data and the DOMINO algorithm, variants in *INTS11* are likely to cause a recessively inherited disease.

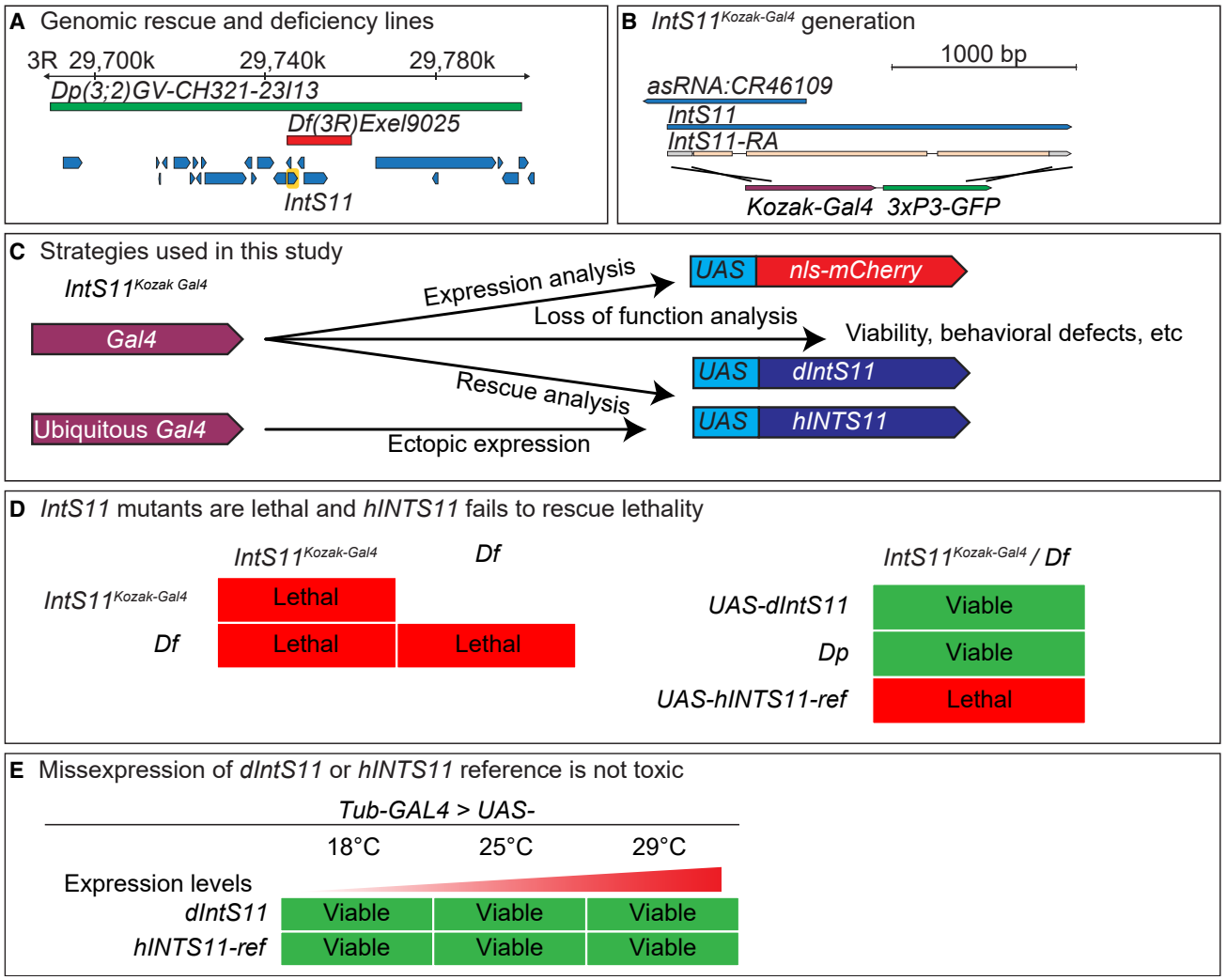


Figure 2. Loss-of-function mutations in *IntS11* in flies cause lethality

(A) Genomic location of the genomic duplication (Dp) was used to rescue *IntS11* mutants, and deficiency construct (Df) was used to uncover the gene for complementation analysis.

(B) Strategy to generate the *Kozak-Gal4* insertion in *dIntS11* locus. Using CRISPR-Cas9-mediated genome editing, we inserted the *Kozak-Gal4* cassette into the *IntS11* locus while removing the entire coding region of the gene.

(C) Strategy to study the effects of variant *IntS11* in flies. Combining $IntS11^{Kozak\text{-}Gal4}$ with *UAS-mCherry.NLS* allows the determination of the expression pattern. The *Kozak-Gal4* insertion also generates a null allele to investigate mutant phenotypes and to perform rescue assays using *UAS-dIntS11* or *UAS-hINTS11*. We also ectopically express *dIntS11* or *hINTS11* reference (Ref) using a ubiquitous GAL4 driver to assess the toxicity of overexpression *in vivo*.

(D) The homozygous $IntS11^{Kozak\text{-}Gal4}$ flies are lethal over a genomic deficiency covering the *dIntS11* locus. Reintroduction of *dIntS11* with a Dp construct or *Kozak-Gal4*-driven overexpression of *dIntS11* rescues the lethality phenotype of $IntS11^{Kozak\text{-}Gal4}/Df$ flies, while overexpression of human reference cDNA does not rescue the lethality.

(E) Overexpression of *UAS-dIntS11* or *UAS-hINTS11* does not cause any change in viability. Three different temperatures are used to control the level of overexpression. The expression level of the UAS construct is lowest at 18°C, highest at 29°C, and intermediate at 25°C.

Based on gnomAD,[43] *INTS11* is predicted to be tolerant to LoF with a pLI score[45] of 0 with an observed/expected (o/e) ratio of 0.72. However, we did not find any individuals in gnomAD with a homozygous LoF variant. In addition, *INTS11* is not constrained to missense variation with a *Z* score of 0.54 and an o/e ratio of 0.92 (Figure 1C).

Loss of function of *IntS11* causes lethality in flies

To study the functional consequence of the variants observed in patients, we utilized *Drosophila melanogaster* as a model organism.[46] *INTS11* is evolutionarily conserved across metazoans and has been confirmed to function similarly in fly cells as in humans.[9,10,22] Human INTS11 and fly IntS11 proteins are highly conserved with a DIOPT (DRSC Integrative Ortholog Prediction Tool) score of 13/16 and exhibit a high similarity (81%) and identity (69%) (Figure S4). In flies, *IntS11* has been studied only in large-scale RNAi knockdown studies and *in vitro* cell culture models, and no mutant alleles are available. Using the CRIMIC (CRISPR-Mediated Integration Cassette) technique, we replaced the entire coding region of the fly *IntS11* gene with the Kozak-Gal4 transcriptional activator ($IntS11^{KozakGal4}$) (Figure 2B).

Specifically, using two guide RNAs targeting 5′ and 3′ UTR of the fly gene, the entire coding region was deleted and replaced with *GAL4* using a homology donor plasmid containing the Kozak sequence followed by *GAL4* with a dominant marker (*3xP3-GFP*) flanked by 200 bp of homology to the cut sites.[33] This strategy generates a complete deletion of *IntS11* and simultaneously allows for the expression of the GAL4 transcription factor with the same spatiotemporal pattern of *IntS11*.[47–49] We used the $IntS11^{KozakGal4}$ allele to assess the *IntS11* LoF phenotype, to rescue associated phenotypes with human or fly cDNAs, and to investigate the expression profile of the fly *IntS11* gene (Figure 2C).

The $IntS11^{Kozak\text{-}Gal4}$ allele causes lethality when homozygous or over a deficiency covering the region containing the *IntS11* gene (*Df(3R) Exel9025*).[50] Mutant flies show developmental delay and live for 5 or 6 days but die as L2 larvae. Lethality can be rescued by 120 kb P[acman] genomic BAC rescue (GR) construct (*Dp (3;2) GV-CH321-23I13*)[36] or by driving expression of the fly *UAS-IntS11* cDNA with $IntS11^{Kozak\text{-}Gal4}$ (Figure 2D). These data demonstrate that fly *IntS11* is an essential gene and that the lethality of $IntS11^{Kozak\text{-}Gal4}$/*Df(3R) Exel9025* is indeed due to the specific loss of *IntS11*.

Fly *IntS11* is enriched in neurons and glia in the nervous system

To assess the expression pattern of fly *IntS11*, we crossed the $IntS11^{Kozak\text{-}Gal4}$ allele to *UAS-mCherry-NLS* to label the nuclei of the cells that express *IntS11*. As shown in Figure 3A, *IntS11* is expressed sparsely in the larval and adult CNS. In both stages, *IntS11* expression co-localizes with a subset of Elav (pan-neuronal nuclear marker)-positive and Repo (pan-glial nuclear marker)-positive cells (Figure 3B).

Loss of fly *IntS11* in glia is lethal

Given that the individuals in Table 1 exhibit neurological phenotypes, we knocked down *IntS11* in either neurons or glia. We used two distinct RNAi lines that differed in the region of the *IntS11* mRNA targeted by the dsRNA and drove their expression using the weak, ubiquitous Gal4 driver (*Da-Gal4*) to assess knockdown efficiency. RT-qPCR analysis of *IntS11* transcripts of 2nd instar larvae (*Da-Gal4>UAS-RNAi IntS11*) showed ∼80% reduction in expression in either RNAi line (Figure 4A). We also expressed both RNAis using a stronger ubiquitous driver (*Act-Gal4*) but did not observe any adult flies, further demonstrating the essential nature of IntS11. Surprisingly, a neuronal-specific Gal4 driver (*elav-Gal4*) did not cause any obvious phenotype with either RNAi line (Figure 4C); however, the glial (*repo-Gal4*) driver caused a significant reduction in the number of eclosed flies (Figures 4B and 4C). Moreover, eclosing adult flies with *IntS11* glial knockdown display a short lifespan and reduced climbing speed at 10 days post-eclosion (Figures 4D and 4E). These data indicate that fly *IntS11* has an important function in the glia of the fly CNS.

IntS11-pArg17Leu and *pHis414Tyr* are severe loss-of-function mutations

Although human INTS11 and fly IntS11 share significant amino acid sequence similarity, expression of human *INTS11* using the *Kozak-GAL4 > UAS-hINTS11* flies did not rescue the lethality associated with the *IntS11*-null mutants (Figure 2D). Overexpression of *hINTS11* using a ubiquitous driver (*Tub-GAL4*) does not affect viability or cause any obvious phenotype (Figure 2E). These data indicate that *hINTS11* cannot functionally replace fly *IntS11* and that it does not cause a dominant effect when mis-/overexpressed. Hence, we decided to study the functional consequence of the variants by generating the corresponding mutations in the fly cDNA (Figure 5A).

We assessed the following seven human INTS11 variants: p.Arg23Leu, p.Gly61Ser, p.Leu144Phe, p.Lys402Glu, p.His420Tyr, p.Val521Met, and p.Val557Glu (Figure 5A), corresponding to the fly IntS11 changes: p.Arg17Leu, p.Gly55Ser, p.Leu138Phe, p.Lys396Glu, p.His414Tyr, p.Val517Met, and p.Ile553Glu. Five variants rescue the lethality, while the fly p.Arg17Leu and p.His414Tyr variants did not, suggesting that these two are strong LoF variants (Figure 5A). We then assessed the lifespan of the flies rescued with the variant proteins. Flies expressing the p.Gly55Ser, p.Leu138Phe, p.Lys396Glu, p.Val517Met, and p.IIe553Glu variants in the *Kozak-GAL4* mutant background showed mild but significant reductions in lifespan (Figure 5B). Since most variants rescue lethality and cause a mild but significant lifespan reduction, we assumed they are weak LoF alleles.

Reducing the expression of patient variants reveals more diverse phenotypes

Flies rescued with missense variants express variant proteins driven by the GAL4-UAS binary expression system. This approach leads to much higher expression levels than typically observed for the endogenous gene expression and may therefore obscure the ability to detect mild reductions in function.[47,51] To address this concern, we reduced the expression levels of the IntS11 variant proteins by introducing $GAL80^{ts}$, a temperature-sensitive inhibitor of GAL4.[52–54] $GAL80^{ts}$ binds and inhibits the transcriptional activation domain (AD) of the GAL4 at 18°C but is degraded at 29°C, thus allowing the GAL4-UAS expression system to function (Figure 6A). Intermediate temperatures allow modulation of expression levels driven by the Kozak-GAL4 insertion and hence allow a more careful assessment of phenotypic rescue. We tested the ability of wild-type *IntS11* to rescue phenotypes at three different temperatures: 22°C, 25°C, and 29°C. At 22°C, we did not observe any adult flies with the WT *UAS-IntS11* construct, indicating that IntS11 expression levels at this temperature are insufficient (Figure 6B). However, at 25°C and 29°C, we observe rescue with the wild-type *IntS11* (Figure 6B). Likewise, all five variants (p.Gly55Ser, p.Leu138Phe, p.Lys396Glu, p.Val517Met, and p.Ile553Glu) rescued lethality at 25°C.

All human variants in *INTS11* are associated with severe neurological symptoms such as ID, language delay, and

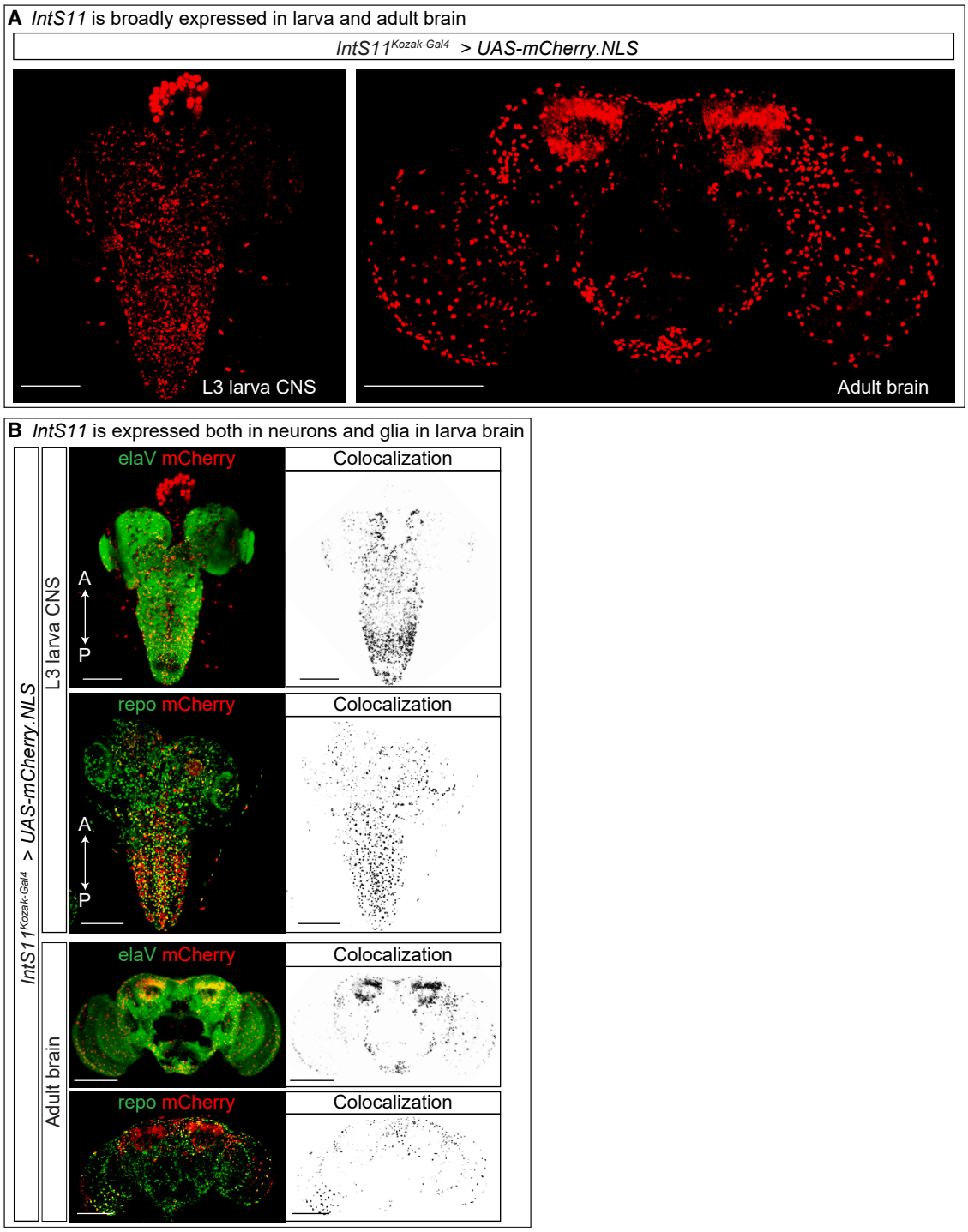
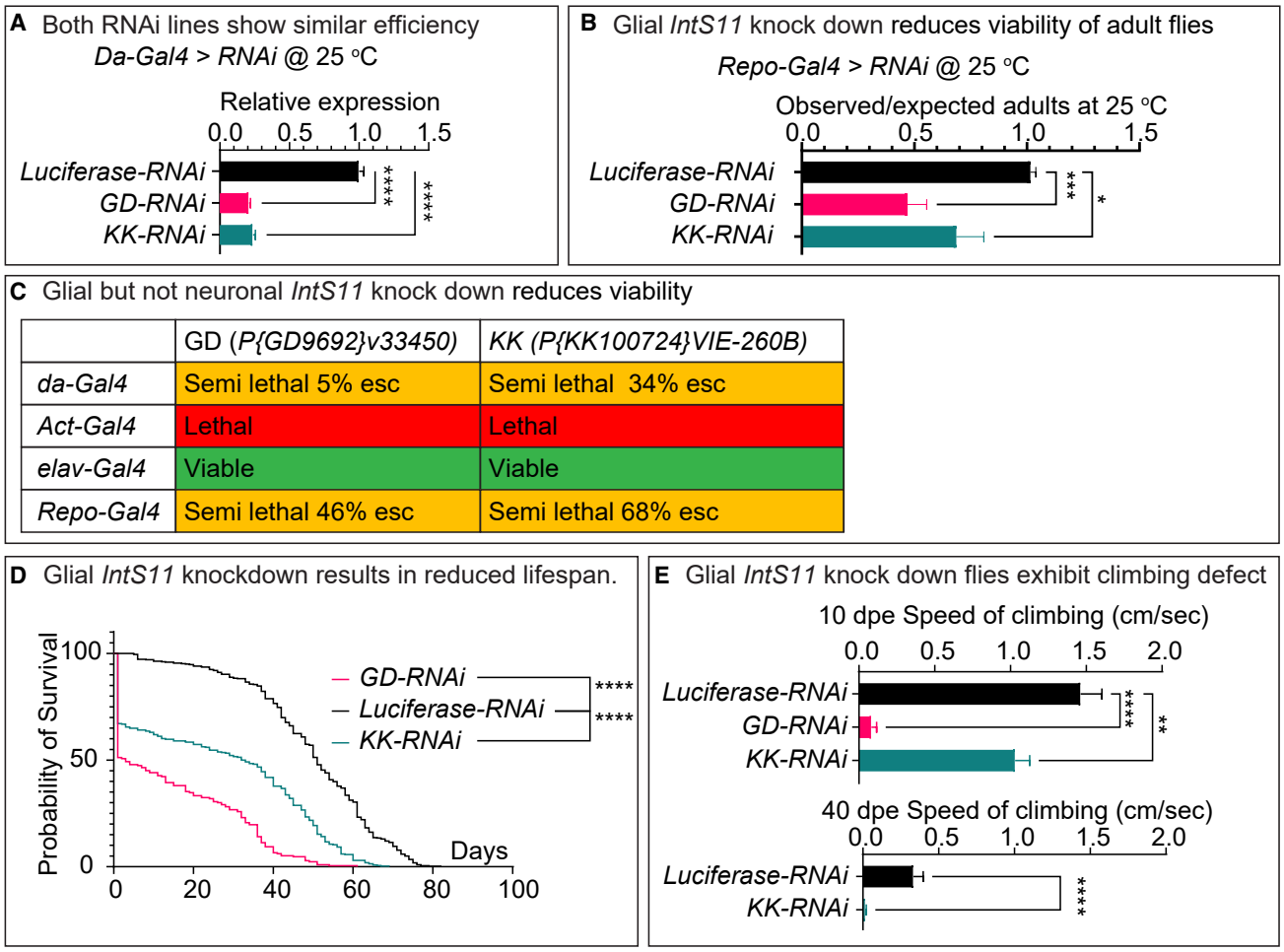


Figure 3. *dIntS11* is expressed in neurons and glia in the CNS

(A) The expression pattern of *dIntS11* in the L3 larval CNS (left) and adult brain (right) is visualized by *dIntS11*$^{Kozak\text{-}Gal4}$-driven expression of *mCherry.NLS*. Scale bars: 100 μm.

(B) L3 larval CNS (top two panels) and adult brain (bottom two panels) samples expressing *dIntS11*$^{Kozak\text{-}Gal4}$-driven *mCherry.NLS* are co-stained with markers for neurons (Elav) or glia (Repo). Colocalization images generated using IMARIS indicate that mCherry is colocalized with both neurons (Elav) or glia (Repo). Scale bars: 100 μm.

motor development defects, and many individuals are non-ambulatory and have seizures. To assess analogous behaviors in flies, we performed a bang-sensitivity assay to determine whether the Kozak-GAL4 flies rescued with variant fly *IntS11* are seizure prone.[37] This assay relies on the mechanical stimulation of flies by vortexing them for 15 s. Flies with defects in brain function display paralysis or seizure-like symptoms after vortexing. The time they take to recover is used to measure the disruption level. Flies rescued with wild-type *IntS11* recover within a few seconds



	GD (*P{GD9692}v33450*)	*KK (P{KK100724}VIE-260B)*
da-Gal4	Semi lethal 5% esc	Semi lethal 34% esc
Act-Gal4	Lethal	Lethal
elav-Gal4	Viable	Viable
Repo-Gal4	Semi lethal 46% esc	Semi lethal 68% esc

Figure 4. Glial *IntS11* knockdown reduces viability, and escapers have a short lifespan with climbing defects

(A) Real-time RT-PCR data show that two different RNAis (GD and KK) driven by a weak ubiquitous driver (*Da-Gal4*) reduce *dIntS11* mRNA levels to about 20% of the endogenous levels in control animals at 25°C. Data are means +SEM. Unpaired test (****$p < 0.0001$).

(B) Glial knockdown of *dIntS11* with both GD- and KK-RNAi reduces the viability of adult flies, as shown by lower-than-expected genotypic survival ratios into adulthood. *Repo-Gal4>UAS-GD-RNAi* or *UAS-KK-RNAi* flies were compared to *Repo-Gal4>UAS-Luciferase-RNAi* (control) flies. Data are means + SEM. Unpaired test (*$p < 0.05$, ***$p < 0.001$).

(C) Summary of lethality phenotype of *IntS11* knockdown using various Gal4 drivers. Both GD- and KK-RNAi lines reduce viability when driven by a weak ubiquitous driver (*Da*-Gal4), and no viable flies were observed when driven by a strong ubiquitous driver (*Act-Gal4*). Pan-neuronal knockdown of *IntS11* with *elav-Gal4*-driven RNAis did not cause any apparent phenotypes, while *repo-Gal4*-driven glial knockdown was semi-lethal for both RNAi lines.

(D) The lifespan of glial *IntS11* knockdown flies. Data are the probability of survival at different ages. Log rank (Mantel-Cox) test (****$p < 0.0001$).

(E) Climbing speed for glial *IntS11* knock down flies at 10- and 40-day post eclosion. Data are means +SEM. Unpaired test (**$p < 0.01$, ****$p < 0.0001$).

with no sign of seizures. However, flies rescued with pGly55Ser, pLeu138Phe, and pIle553Glu variants are paralyzed, uncoordinated, and exhibit a seizure-like phenotype for a significantly longer period at 30 days post eclosion (Figure 6C). Flies rescued with other variants (*p.Lys396Glu* and *p.Val517Met*) also displayed longer recovery times, but the data are not statistically significant.

Next, we investigated locomotor defects by performing a *Drosophila* Activity Monitoring (DAM) assay[55] of adult flies rescued with the variant *IntS11* at 25°C. First, we monitored their activity for five days with 12-h light and 12-h dark cycle. Flies rescued with p.Val517Met and p.Ile553Glu variants displayed reduced locomotor activity throughout the day (Figure 6D). Next, we exposed them to five days of constant darkness, and four variants (p.Gly55Ser, p.Leu138Phe, p.Val517Met, and p.Ile553Glu) displayed lower locomotor activity (Figure 6D). Hence, all but the p.Lys396Glu variant (p.Lys402Glu in human INTS11) cause reduced locomotion.

Discussion

INTS11 is considered a critical subunit of Integrator as it houses the RNA endonuclease activity, but *INTS11* variants have not previously been associated with human disease. Here we describe a cohort of 15 individuals with bi-allelic variants in *INTS11* that cause a neurodevelopmental disorder

A Missense variants and their corresponding amino acids on fly *IntS11*

Human	Fly	$\frac{IntS11^{Kozak\text{-}Gal4}}{Df(3R)Exel9025}$ Rescue
pArg23Leu	pArg17Leu	Lethal
pGly61Ser	pGly55Ser	Viable
pLeu144Phe	pLeu138Phe	Viable
pLys402Glu	pLys396Glu	Viable
pHis420Tyr	pHis414Tyr	Lethal
pVal521Met	pVal517Met	Viable
pVal557Glu	pIle553Glu	Viable

B Mutant flies rescued with variant cDNAs show reduced lifespan

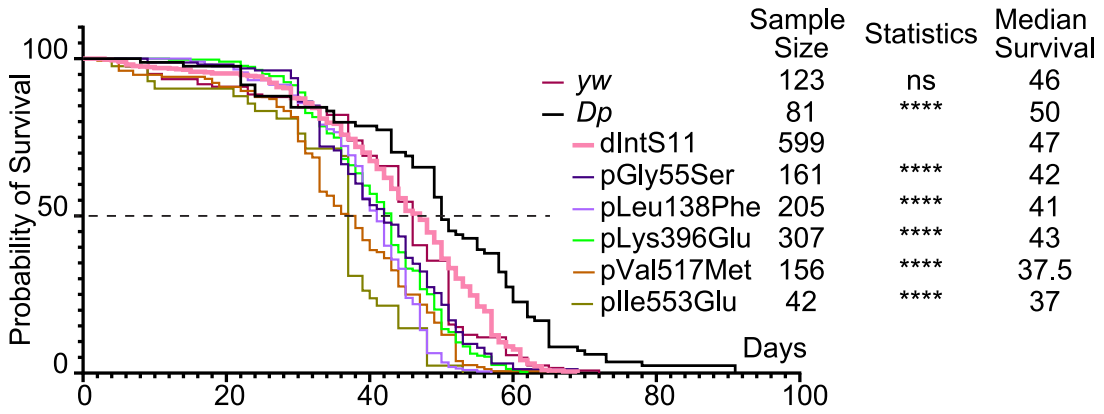


Figure 5. Two of the variants are strong loss-of-function alleles and five of the variants are weak loss-of-function alleles
(A) Summary of missense variants, their fly counterparts, and their effect on the lethality phenotype of $dIntS11^{Kozak\text{-}Gal4}$. *pArg17Leu* and *pHis414Tyr* variants do not rescue lethality, arguing that they are strong loss-of-function alleles.
(B) The lifespan of flies rescued with wild-type or variant cDNA. Data are the probability of survival at different ages. Log rank (Mantel-Cox) test (****$p < 0.0001$).

characterized by DD, ID, generalized hypotonia, and cerebral and cerebellar atrophy. Neurodevelopmental processes were found to be impaired in all individuals (15/15) and they presented with defective motor development and speech. Of those with speech issues, it was noted that they used few words or only short phrases. ID was severe in most individuals. Generalized hypotonia was observed in a majority of this cohort (78%), consistent with phenotypic descriptions from individuals with other Integrator complex variants.[28–30] Brain MRI changes are present in all individuals for whom we have data, but this is variable among individuals, with cerebral progressive cortical atrophy, delayed myelination, and pontocerebellar hypoplasia being the most common findings (Tables 1 and S1). The motor delay was also variable, with eight individuals never achieving independent ambulation. Those who did achieve independent ambulation did so late. Further, an ataxic gait was commonly observed in individuals with ambulation. Optic atrophy, visual impairment, and seizures documented in the most severe cases are believed to be the result of progressive degenerative processes involving the loss and/or dysfunction of brain cells (Table 1 and supplemental materials). A larger cohort of affected individuals will be needed to confirm the recurrence of some rare symptoms.

The Integrator complex comprises 15 subunits and is critical for regulating gene expression at nearly all RNAPII-transcribed loci.[11] Only 3 of the 15 Integrator complex subunits have previously been associated with human disease. Interestingly, individuals with bi-allelic variants in *INTS1* and *INTS8* display features similar to those observed in

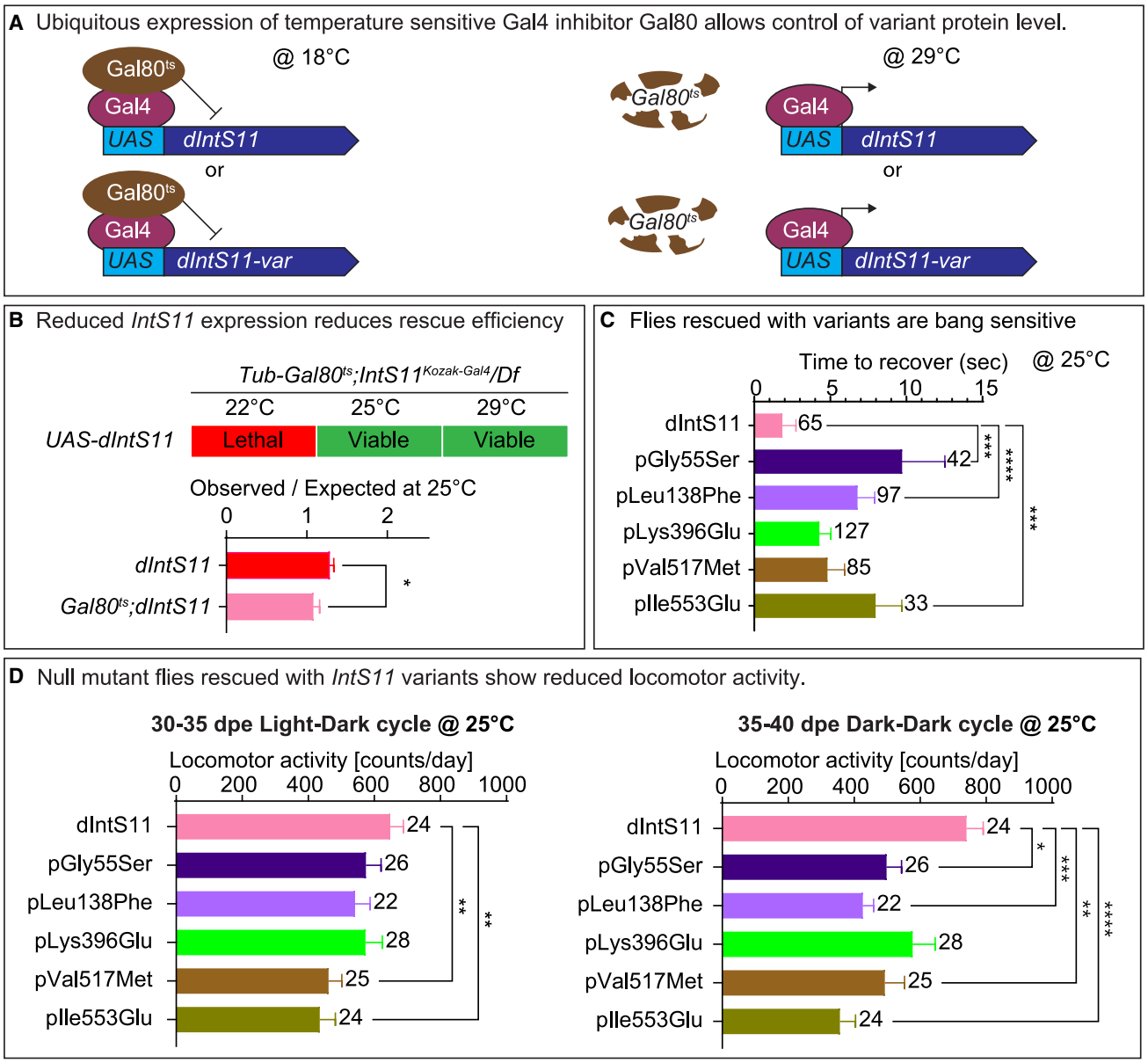


Figure 6. Reduced level of variant cDNA expression reveals locomotor defects in rescue flies

(A) Schematic representation of Gal80^{ts}-mediated Gal4-UAS system inhibition. Gal80^{ts} binds to Gal4 at a restrictive temperature (18°C) and blocks the transcriptional activation domain of Gal4, leading to the inhibition of downstream UAS constructs. At permissive temperature (29°C), Gal80^{ts} degrades, and Gal4 can drive the expression of downstream UAS constructs. Intermediate temperatures allow fine-tuning of UAS construct expression.

(B) Summary of lethality phenotype rescued with wild-type *dIntS11* at different temperatures and the observed/expected ratios at 25°C. Data are means + SEM. Unpaired test (*p < 0.05).

(C) Bang sensitivity of null mutant flies rescued with *IntS11* variants at 25°C measured using the time to recover after 15 s of vortex. Kruskal-Wallis test was followed by a Dunn's test. Data are means + SEM (*p < 0.05, **p < 0.01).

(D) Locomotor activity of null mutant flies rescued with *IntS11* variants at 25°C measured by DAM assay. Kruskal-Wallis test was followed by a Dunn's test. Data are means +SEM (*p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001).

individuals with variants in *INTS11*, including ID, DD with delayed or absent speech development, hypotonia, motor impairment, as well as some individuals with seizures, similar craniofacial abnormalities, and visual impairment (Table S1). Consistently, individuals with variants in *INTS13* show mainly craniofacial malformations, ID, and delayed language development, and some exhibit mild cerebellar atrophy.[30] The clinical picture is believed to resemble the orofaciodigital (OFC2) syndrome (MIM: 252100) even though several features are also recognizable in *INTS11*-affected individuals of this cohort. Indeed, beside DD/ID and the abnormalities of the brain anatomy, some craniofacial features (i.e., microcephaly, hypertelorism, epicanthal folds, broad nasal root, and cleft lips) are also characteristic of the *INTS11*-related clinical portrait. However, individuals with variants in *INTS13* do not have prominent movement

defects, hypotonia, or seizures. This argues that the disruption of different subunits may have slightly different consequences in distinct tissues.

To date, all LoF alleles of Integrator complex subunits in various model organisms have been shown to cause homozygous lethality.[56–61] Here we show that loss of *IntS11* in flies also causes lethality during larval development. We did not expect to observe that *IntS11* is not ubiquitously expressed in flies. Moreover, it is surprising that neuronal knockdown does not cause any observable phenotype, suggesting that Integrator complex function in neurons may not be essential or only very low levels of IntS11 are required. However, reducing glial expression leads to reduced survival and a severe reduction in lifespan and climbing speed. This argues that a robust function of the complex in glia is important. Further studies are needed to understand the basis of the difference between these two cell types.

Fly *IntS11* mutants die at larval stages and the human cDNA did not rescue the lethality associated with loss of fly *IntS11*. One of the possible reasons is the lower conservation level observed between the CTD domain of human and fly proteins. The CTD of INTS11 mediates the heterotrimer formation with INTS4 and INTS9 to create what has been termed the "Integrator Cleavage Module."[17–21] Similarly, the CTD of INTS9 also shows lower levels of conservation with fly IntS9,[17] which argues that co-evolution of the INTS9 and INTS11 interaction domains occurred. Hence, human INTS11 may not be able to form a functional complex with the rest of the fly Integrator Cleavage Module. Additional studies are required to test the interaction between human INTS11 and fly IntS9 and IntS4. When introduced to fly protein, two missense variants (p.Arg17Leu and p.His414Tyr) fail to rescue the lethality associated with *IntS11* loss. The p.His414Tyr variant is particularly intriguing because this histidine is involved in the coordination of zinc in the INTS11 active site and suggest a critical role for RNA cleavage in brain development. Although all other variants tested (p.Gly55Ser, p.Leu138Phe, pLys396Glu, p.Val517Met, and p.Ile553Glu) rescue lethality, they all exhibit a significant reduction in lifespan. It is not yet understood how Integrator function is connected to aging, so it is not obvious why these flies die early. Given that the rescue strategy relies on overexpression based on the Gal4-UAS binary expression, the overexpression of the variants may mask the phenotype associated with the mild LoF of these alleles. We, therefore, reduced expression levels of variants that rescue lethality with Gal80^{ts}, a temperature-sensitive inhibitor of Gal4 activity. Upon reduction of the Gal4 activity and performing behavioral assays of eclosed adult flies, we observed bang sensitivity and aberrant behavior in DAM assays for five variants (p.Gly55Ser, p.Leu138Phe, pLys396Glu, p.Val517Met, and p.Ile553Glu), mild LoF alleles of *IntS11*. In summary, two variants correspond to severe LoF alleles and five are mild LoF alleles of *IntS11*.

In gnomAD, no individuals are reported to have a homozygous loss of *INTS11*.[43] However, the gene is tolerant of LoF mutations based on the pLI score[45] of 0. Hence, the loss of one copy of the gene is not sufficient to cause noticeable clinical signs. We, therefore, argue that the individuals in our cohort have inherited a strong LoF allele and a mild LoF allele. Indeed, 4/15 individuals (subjects 1, 2, 5, and 9) with *INTS11* variants contain a frameshift or a stop gain variant, resulting in a truncated protein expression, and a missense variant (Table 1). Another 3/15 individuals (subjects 13–15) are homozygous for p.Arg23Leu, a severe LoF allele, and these individuals died at an early age. 2/15 individuals (subjects 3 and 4) carry the p.His420Tyr severe LOF variant and a mild missense variant (p.Leu144-Phe). Lastly, one individual (subject 6) has two mild LoF alleles (p.Gly61Ser and p.Val521Met). Finally, 8/15 individuals (subjects 1, 2, 7, 8, 9, 10, 11, and 12) carry missense variants of unknown significance.

Recent studies have documented a novel physical and genetic interaction between the Integrator complex and *BRAT1*, **BR**CA1-**A**ssociated A**T**M activator 1.[62] BRAT1 interacts with and stabilizes INTS11. Variants in *BRAT1* were previously associated with three different disorders based on the severity of the symptoms. The strongest forms cause lethal neonatal rigidity and multifocal seizure syndrome (RMFSL [MIM: 614498]), characterized by microcephaly, hypertonia, epilepsy, seizures, and death within two years of birth.[63–65] Milder forms include neurodevelopmental disorder with cerebellar atrophy with or without seizures (NEDCAS [MIM: 618056]), epilepsy of infancy with migrating focal seizures (EIMFS), and congenital ataxia (CA).[66–69] The mildest forms include non-progressive cerebellar ataxia (NPCA).[70,71] The similarities of some symptoms observed in individuals with *BRAT1* and *INTS11* variants, including cerebellar atrophy, ataxia, and cognitive impairment, suggest that some *INTS11* variants affect this interaction. Further studies are required to determine molecular mechanisms of how the numerous *INTS11* variants disrupt function.

In summary, our findings show that bi-allelic variants in *INTS11* cause severe developmental delay, intellectual disability, language delay, and motor development disorders with brain MRI defects. Given that the human genome databases do not contain healthy individuals with homozygous loss of *INTS11* and that loss of *IntS11* is lethal in flies, fish, mouse, and worms, it is highly likely that *INTS11* is an essential gene in humans. Affected individuals with tested variants in *INTS11* typically inherit a strong LoF variant and a milder variant. Further studies examining the effect of the variants on the biochemical function of *INTS11* will lead to a better understanding of the pathology of *INTS11*-related disorders.

Consortia

The members of the Undiagnosed Diseases Network are Maria T. Acosta, Margaret Adam, David R. Adams, Justin Alvey, Laura Amendola, Ashley Andrews, Euan A. Ashley, Mahshid S. Azamian, Carlos A. Bacino, Guney Bademci,

Ashok Balasubramanyam, Dustin Baldridge, Jim Bale, Michael Bamshad, Deborah Barbouth, Pinar Bayrak-Toydemir, Anita Beck, Alan H. Beggs, Edward Behrens, Gill Bejerano, Hugo J. Bellen, Jimmy Bennet, Beverly Berg-Rood, Jonathan A. Bernstein, Gerard T. Berry, Anna Bican, Stephanie Bivona, Elizabeth Blue, John Bohnsack, Devon Bonner, Lorenzo Botto, Brenna Boyd, Lauren C. Briere, Elly Brokamp, Gabrielle Brown, Elizabeth A. Burke, Lindsay C. Burrage, Manish J. Butte, Peter Byers, William E. Byrd, John Carey, Olveen Carrasquillo, Thomas Cassini, Ta Chen Peter Chang, Sirisak Chanprasert, Hsiao-Tuan Chao, Gary D. Clark, Terra R. Coakley, Laurel A. Cobban, Joy D. Cogan, Matthew Coggins, F. Sessions Cole, Heather A. Colley, Cynthia M. Cooper, Heidi Cope, William J. Craigen, Andrew B. Crouse, Michael Cunningham, Precilla D'Souza, Hongzheng Dai, Surendra Dasari, Joie Davis, Jyoti G. Dayal, Matthew Deardorff, Esteban C. Dell'Angelica, Katrina Dipple, Daniel Doherty, Naghmeh Dorrani, Argenia L. Doss, Emilie D. Douine, Laura Duncan, Dawn Earl, David J. Eckstein, Lisa T. Emrick, Christine M. Eng, Cecilia Esteves, Marni Falk, Liliana Fernandez, Elizabeth L. Fieg, Paul G. Fisher, Brent L. Fogel, Irman Forghani, William A. Gahl, Ian Glass, Bernadette Gochuico, Rena A. Godfrey, Katie Golden-Grant, Madison P. Goldrich, Alana Grajewski, Irma Gutierrez, Don Hadley, Sihoun Hahn, Rizwan Hamid, Kelly Hassey, Nichole Hayes, Frances High, Anne Hing, Fuki M. Hisama, Ingrid A. Holm, Jason Hom, Martha Horike-Pyne, Alden Huang, Yong Huang, Wendy Introne, Rosario Isasi, Kosuke Izumi, Fariha Jamal, Gail P. Jarvik, Jeffrey Jarvik, Suman Jayadev, Orpa Jean-Marie, Vaidehi Jobanputra, Lefkothea Karaviti, Jennifer Kennedy, Shamika Ketkar, Dana Kiley, Gonench Kilich, Shilpa N. Kobren, Isaac S. Kohane, Jennefer N. Kohler, Deborah Krakow, Donna M. Krasnewich, Elijah Kravets, Susan Korrick, Mary Koziura, Seema R. Lalani, Byron Lam, Christina Lam, Grace L. LaMoure, Brendan C. Lanpher, Ian R. Lanza, Kimberly LeBlanc, Brendan H. Lee, Roy Levitt, Richard A. Lewis, Pengfei Liu, Xue Zhong Liu, Nicola Longo, Sandra K. Loo, Joseph Loscalzo, Richard L. Maas, Ellen F. Macnamara, Calum A. MacRae, Valerie V. Maduro, Rachel Mahoney, Bryan C. Mak, May Christine V. Malicdan, Laura A. Mamounas, Teri A. Manolio, Rong Mao, Kenneth Maravilla, Ronit Marom, Gabor Marth, Beth A. Martin, Martin G. Martin, Julian A. Martínez-Agosto, Shruti Marwaha, Jacob McCauley, Allyn McConkie-Rosell, Alexa T. McCray, Elisabeth McGee, Heather Mefford, J. Lawrence Merritt, Matthew Might, Ghayda Mirzaa, Eva Morava, Paolo M. Moretti, Mariko Nakano-Okuno, Stan F. Nelson, John H. Newman, Sarah K. Nicholas, Deborah Nickerson, Shirley Nieves-Rodriguez, Donna Novacic, Devin Oglesbee, James P. Orengo, Laura Pace, Stephen Pak, J. Carl Pallais, Christina GS. Palmer, Jeanette C. Papp, Neil H. Parker, John A. Phillips III, Jennifer E. Posey, Lorraine Potocki, Barbara N. Pusey, Aaron Quinlan, Wendy Raskind, Archana N. Raja, Deepak A. Rao, Anna Raper, Genecee Renteria, Chloe M. Reuter, Lynette Rives, Amy K. Robertson, Lance H. Rodan, Jill A. Rosenfeld, Natalie Rosenwasser, Francis Rossignol, Maura Ruzhnikov, Ralph Sacco, Jacinda B. Sampson, Mario Saporta, Judy Schaechter, Timothy Schedl, Kelly Schoch, C. Ron Scott, Daryl A. Scott, Vandana Shashi, Jimann Shin, Edwin K. Silverman, Janet S. Sinsheimer, Kathy Sisco, Edward C. Smith, Kevin S. Smith, Emily Solem, Lilianna Solnica- Krezel, Ben Solomon, Rebecca C. Spillmann, Joan M. Stoler, Jennifer A. Sullivan, Kathleen Sullivan, Angela Sun, Shirley Sutton, David A. Sweetser, Virginia Sybert, Holly K. Tabor, Amelia L. M. Tan, Queenie K.-G. Tan, Mustafa Tekin, Fred Telischi, Willa Thorson, Cynthia J. Tifft, Camilo Toro, Alyssa A. Tran, Brianna M. Tucker, Tiina K. Urv, Adeline Vanderver, Matt Velinder, Dave Viskochil, Tiphanie P. Vogel, Colleen E. Wahl, Melissa Walker, Stephanie Wallace, Nicole M. Walley, Jennifer Wambach, Jijun Wan, Lee-kai Wang, Michael F. Wangler, Patricia A. Ward, Daniel Wegner, Monika Weisz-Hubshman, Mark Wener, Tara Wenger, Katherine Wesseling Perry, Monte Westerfield, Matthew T. Wheeler, Jordan Whitlock, Lynne A. Wolfe, Kim Worley, Changrui Xiao, Shinya Yamamoto, John Yang, Diane B. Zastrow, Zhe Zhang, Chunli Zhao, Stephan Zuchner.

Supplemental information

Supplemental information can be found online at https://doi.org/10.1016/j.ajhg.2023.03.012.

Acknowledgments

We would like to thank the families who participated in this study. We thank Hongling Pan for injections to create transgenic flies and Zelha Nil for input on the manuscript. We thank the Bloomington Drosophila Stock Center (BDSC) for fly stocks and the Developmental Studies Hybridoma Bank for antibodies. This work was supported by the Model Organisms Screening Center of the UDN through U54 NS093793 (NINDS), the Office of Research Infrastructure Programs of the NIH (awards R01 AG073260, R24 OD022005, and R24 OD031447), the Huffington Foundation, the Jan and Dan Duncan Neurological Research Institute at Texas Children's Hospital to H.J.B., the Baylor College of Medicine IDDRC P50HD103555 from the Eunice Kennedy Shriver National Institute of Child Health and Human Development for use of the Microscopy Core facilities, the NIHR (M.A.K.: NIHR-RP-2016-07-019, funding D.S.), Sir Jules Thorn Trust (M.A.K.: 17JTA), and Rosetrees Trust (M.A.K.); the Italian Ministry of Health (RCR-2021-23671215 and 5x1000_2019 to M.T.). E.J.W. acknowledges salary support from NIH R01GM134539. Sequencing and analysis of family 5 were provided by the Broad Institute of MIT and Harvard Center for Mendelian Genomics (Broad CMG) and was funded by the National Human Genome Research Institute, the National Eye Institute, and the National Heart, Lung, and Blood Institute grant UM1 HG008900 and in part by National Human Genome Research Institute grant R01 HG009141. C.A.W. is supported by a grant from the NINDS (R01 NS035129) and is an Investigator of the Howard Hughes Medical Institute.

Declaration of interests

The authors declare no competing interests.

Received: January 5, 2023
Accepted: March 18, 2023
Published: April 12, 2023

Web resources

CADD, https://cadd.gs.washington.edu/
DIOPT, https://www.flyrnai.org/diopt
ENSEMBL Variant Effect Predictor, https://grch37.ensembl.org/Homo_sapiens/Tools/VEP
GenBank, https://www.ncbi.nlm.nih.gov/genbank/
gnomAD, http://gnomad.broadinstitute.org/
IMARIS 9.9 Oxford Instruments, https://imaris.oxinst.com
MARRVEL, http://www.marrvel.org/
OMIM, http://www.omim.org/

References

1. Kirstein, N., Gomes Dos Santos, H., Blumenthal, E., and Shiekhattar, R. (2021). The Integrator complex at the crossroad of coding and noncoding RNA. Curr. Opin. Cell Biol. *70*, 37–43.
2. Nojima, T., and Proudfoot, N.J. (2022). Mechanisms of lncRNA biogenesis as revealed by nascent transcriptomics. Nat. Rev. Mol. Cell Biol. *23*, 389–406.
3. Baillat, D., Hakimi, M.A., Näär, A.M., Shilatifard, A., Cooch, N., and Shiekhattar, R. (2005). Integrator, a multiprotein mediator of small nuclear RNA processing, associates with the C-terminal repeat of RNA polymerase II. Cell *123*, 265–276.
4. Jurica, M.S., and Moore, M.J. (2003). Pre-mRNA splicing: awash in a sea of proteins. Mol. Cell *12*, 5–14.
5. Lai, F., Gardini, A., Zhang, A., and Shiekhattar, R. (2015). Integrator mediates the biogenesis of enhancer RNAs. Nature *525*, 399–403.
6. Rubtsova, M.P., Vasilkova, D.P., Moshareva, M.A., Malyavko, A.N., Meerson, M.B., Zatsepin, T.S., Naraykina, Y.V., Beletsky, A.V., Ravin, N.V., and Dontsova, O.A. (2019). Integrator is a key component of human telomerase RNA biogenesis. Sci. Rep. *9*, 1701.
7. Cazalla, D., Xie, M., and Steitz, J.A. (2011). A primate herpesvirus uses the integrator complex to generate viral microRNAs. Mol. Cell *43*, 982–992.
8. Xie, M., Zhang, W., Shu, M.D., Xu, A., Lenis, D.A., DiMaio, D., and Steitz, J.A. (2015). The host integrator complex acts in transcription-independent maturation of herpesvirus microrna 3′ ends. Genes Dev. *29*, 1552–1564.
9. Elrod, N.D., Henriques, T., Huang, K.L., Tatomer, D.C., Wilusz, J.E., Wagner, E.J., and Adelman, K. (2019). The integrator complex attenuates promoter-proximal transcription at protein-coding genes. Mol. Cell *76*, 738–752.e7.
10. Huang, K.L., Jee, D., Stein, C.B., Elrod, N.D., Henriques, T., Mascibroda, L.G., Baillat, D., Russell, W.K., Adelman, K., and Wagner, E.J. (2020). Integrator recruits protein phosphatase 2A to prevent pause release and facilitate transcription termination. Mol. Cell *80*, 345–358.e9.
11. Stein, C.B., Field, A.R., Mimoso, C.A., Zhao, C., Huang, K.L., Wagner, E.J., and Adelman, K. (2022). Integrator endonuclease drives promoter-proximal termination at all RNA polymerase II-transcribed loci. Mol. Cell *82*, 4232–4245.e11.
12. Vervoort, S.J., Welsh, S.A., Devlin, J.R., Barbieri, E., Knight, D.A., Offley, S., Bjelosevic, S., Costacurta, M., Todorovski, I., Kearney, C.J., et al. (2021). The PP2A-Integrator-CDK9 axis fine-tunes transcription and can be targeted therapeutically in cancer. Cell *184*, 3143–3162.e32.
13. Zheng, H., Qi, Y., Hu, S., Cao, X., Xu, C., Yin, Z., Chen, X., Li, Y., Liu, W., Li, J., et al. (2020). Identification of Integrator-PP2A complex (INTAC), an RNA polymerase II phosphatase. Science *370*, eabb5872.
14. Lykke-Andersen, S., Žumer, K., Molska, E.Š., Rouvière, J.O., Wu, G., Demel, C., Schwalb, B., Schmid, M., Cramer, P., and Jensen, T.H. (2021). Integrator is a genome-wide attenuator of non-productive transcription. Mol. Cell *81*, 514–529.e6.
15. Fianu, I., Chen, Y., Dienemann, C., Dybkov, O., Linden, A., Urlaub, H., and Cramer, P. (2021). Structural basis of Integrator-mediated transcription regulation. Science *374*, 883–887.
16. Callebaut, I., Moshous, D., Mornon, J.P., and De Villartay, J.P. (2002). Metallo-β-lactamase fold within nucleic acids processing enzymes: The β-CASP family. Nucleic Acids Res. *30*, 3592–3601.
17. Wu, Y., Albrecht, T.R., Baillat, D., Wagner, E.J., and Tong, L. (2017). Molecular basis for the interaction between Integrator subunits IntS9 and IntS11 and its functional importance. Proc. Natl. Acad. Sci. USA *114*, 4394–4399.
18. Albrecht, T.R., and Wagner, E.J. (2012). snRNA 3′ end formation requires heterodimeric association of integrator subunits. Mol. Cell Biol. *32*, 1112–1123.
19. Albrecht, T.R., Shevtsov, S.P., Wu, Y., Mascibroda, L.G., Peart, N.J., Huang, K.L., Sawyer, I.A., Tong, L., Dundr, M., and Wagner, E.J. (2018). Integrator subunit 4 is a 'Symplekin-like' scaffold that associates with INTS9/11 to form the Integrator cleavage module. Nucleic Acids Res. *46*, 4241–4255.
20. Lin, M.H., Jensen, M.K., Elrod, N.D., Huang, K.L., Welle, K.A., Wagner, E.J., and Tong, L. (2022). Inositol hexakisphosphate is required for Integrator function. Nat. Commun. *13*, 5742.
21. Pfleiderer, M.M., and Galej, W.P. (2021). Structure of the catalytic core of the Integrator complex. Mol. Cell *81*, 1246–1259.e8.
22. Ezzeddine, N., Chen, J., Waltenspiel, B., Burch, B., Albrecht, T., Zhuo, M., Warren, W.D., Marzluff, W.F., and Wagner, E.J. (2011). A subset of drosophila integrator proteins is essential for efficient U7 snRNA and spliceosomal snRNA 3′-end formation. Mol. Cell Biol. *31*, 328–341.
23. Hu, Y., Flockhart, I., Vinayagam, A., Bergwitz, C., Berger, B., Perrimon, N., and Mohr, S.E. (2011). An integrative approach to ortholog prediction for disease-focused and other functional studies. BMC Bioinf. *12*, 357.
24. Chen, J., Ezzeddine, N., Waltenspiel, B., Albrecht, T.R., Warren, W.D., Marzluff, W.F., and Wagner, E.J. (2012). An RNAi screen identifies additional members of the Drosophila Integrator complex and a requirement for cyclin C/Cdk8 in snRNA 3 '-end formation. RNA *18*, 2148–2156.
25. Matera, A.G., Terns, R.M., and Terns, M.P. (2007). Non-coding RNAs: lessons from the small nuclear and small nucleolar RNAs. Nat. Rev. Mol. Cell Biol. *8*, 209–220.
26. Neumüller, R.A., Richter, C., Fischer, A., Novatchkova, M., Neumüller, K.G., and Knoblich, J.A. (2011). Genome-wide analysis of self-renewal in Drosophila neural stem cells by transgenic RNAi. Cell Stem Cell *8*, 580–593.
27. Neely, G.G., Kuba, K., Cammarato, A., Isobe, K., Amann, S., Zhang, L., Murata, M., Elmén, L., Gupta, V., Arora, S., et al. (2010). A global in vivo Drosophila RNAi screen identifies NOT3 as a conserved regulator of heart function. Cell *141*, 142–153.
28. Krall, M., Htun, S., Schnur, R.E., Brooks, A.S., Baker, L., de Alba Campomanes, A., Lamont, R.E., Gripp, K.W., Care 4 Rare Canada Consortium, and Schneidman-Duhovny, D., et al. (2019). Biallelic sequence variants in INTS1 in patients with

developmental delays, cataracts, and craniofacial anomalies. Eur. J. Hum. Genet. *27*, 582–593.
29. Oegema, R., Baillat, D., Schot, R., van Unen, L.M., Brooks, A., Kia, S.K., Hoogeboom, A.J.M., Xia, Z., Li, W., Cesaroni, M., et al. (2017). Human mutations in integrator complex subunits link transcriptome integrity to brain development. PLoS Genet. *13*, e1006923.
30. Mascibroda, L.G., Shboul, M., Elrod, N.D., Colleaux, L., Hamamy, H., Huang, K.L., Peart, N., Singh, M.K., Lee, H., Merriman, B., et al. (2022). INTS13 variants causing a recessive developmental ciliopathy disrupt assembly of the Integrator complex. Nat. Commun. *13*, 6054.
31. Sobreira, N., Schiettecatte, F., Valle, D., and Hamosh, A. (2015). GeneMatcher: a matching tool for connecting investigators with an interest in the same gene. Hum. Mutat. *36*, 928–930.
32. Firth, H.V., Richards, S.M., Bevan, A.P., Clayton, S., Corpas, M., Rajan, D., Van Vooren, S., Moreau, Y., Pettett, R.M., and Carter, N.P. (2009). DECIPHER: Database of Chromosomal Imbalance and Phenotype in Humans Using Ensembl Resources. Am. J. Hum. Genet. *84*, 524–533.
33. Kanca, O., Zirin, J., Hu, Y., Tepe, B., Dutta, D., Lin, W.W., Ma, L., Ge, M., Zuo, Z., Liu, L.P., et al. (2022). An expanded toolkit for Drosophila gene tagging using synthesized homology donor constructs for CRISPR mediated homologous recombination. Elife. Published online June 2022. https://doi.org/10.7554/eLife.76077.
34. Venken, K.J.T., Carlson, J.W., Schulze, K.L., Pan, H., He, Y., Spokony, R., Wan, K.H., Koriabine, M., de Jong, P.J., White, K.P., et al. (2009). Versatile P[acman] BAC libraries for transgenesis studies in Drosophila melanogaster. Nat. Methods *6*, 431–434.
35. Bischof, J., Maeda, R.K., Hediger, M., Karch, F., and Basler, K. (2007). An optimized transgenesis system for Drosophila using germ-line-specific φC31 integrases. Proc. Natl. Acad. Sci. USA *104*, 3312–3317.
36. Venken, K.J.T., He, Y., Hoskins, R.A., and Bellen, H.J. (2006). P[acman]: A BAC transgenic platform for targeted insertion of large DNA fragments in D-melanogaster. Science *314*, 1747–1751.
37. Burg, M.G., and Wu, C.F. (2012). Mechanical and temperature stressor-induced seizure-and-paralysis behaviors in Drosophila bang-sensitive mutants. J. Neurogenet. *26*, 189–197.
38. Madabattula, S.T., Strautman, J.C., Bysice, A.M., O'Sullivan, J.A., Androschuk, A., Rosenfelt, C., Doucet, K., Rouleau, G., and Bolduc, F. (2015). Quantitative analysis of climbing defects in a drosophila model of neurodegenerative disorders. J. Vis. Exp. *100*, e52741.
39. Kircher, M., Witten, D.M., Jain, P., O'Roak, B.J., Cooper, G.M., and Shendure, J. (2014). A general framework for estimating the relative pathogenicity of human genetic variants. Nat. Genet. *46*, 310–315.
40. Rentzsch, P., Witten, D., Cooper, G.M., Shendure, J., and Kircher, M. (2019). CADD: predicting the deleteriousness of variants throughout the human genome. Nucleic Acids Res. *47*, D886–D894.
41. Rentzsch, P., Schubach, M., Shendure, J., and Kircher, M. (2021). CADD-Splice-improving genome-wide variant effect prediction using deep learning-derived splice scores. Genome Med. *13*, 31.
42. Adzhubei, I.A., Schmidt, S., Peshkin, L., Ramensky, V.E., Gerasimova, A., Bork, P., Kondrashov, A.S., and Sunyaev, S.R. (2010). A method and server for predicting damaging missense mutations. Nat. Methods *7*, 248–249.
43. Karczewski, K.J., Francioli, L.C., Tiao, G., Cummings, B.B., Alföldi, J., Wang, Q., Collins, R.L., Laricchia, K.M., Ganna, A., Birnbaum, D.P., et al. (2020). The mutational constraint spectrum quantified from variation in 141,456 humans. Nature *581*, 434–443.
44. Wang, J., Al-Ouran, R., Hu, Y., Kim, S.Y., Wan, Y.W., Wangler, M.F., Yamamoto, S., Chao, H.T., Comjean, A., Mohr, S.E., et al. (2017). MARRVEL: integration of human and model organism genetic resources to facilitate functional annotation of the human genome. Am. J. Hum. Genet. *100*, 843–853.
45. Lek, M., Karczewski, K.J., Minikel, E.V., Samocha, K.E., Banks, E., Fennell, T., O'Donnell-Luria, A.H., Ware, J.S., Hill, A.J., Cummings, B.B., et al. (2016). Analysis of protein-coding genetic variation in 60,706 humans. Nature *536*, 285–291.
46. Oriel, C., and Lasko, P. (2018). Recent developments in using drosophila as a model for human genetic disease. Int. J. Mol. Sci. *19*, 2041.
47. Lee, P.T., Zirin, J., Kanca, O., Lin, W.W., Schulze, K.L., Li-Kroeger, D., Tao, R., Devereaux, C., Hu, Y., Chung, V., et al. (2018). A gene-specific T2A-GAL4 library for drosophila. Elife *7*, 355744–e35624.
48. Nagarkar-Jaiswal, S., Deluca, S.Z., Lee, P.T., Lin, W.W., Pan, H., Zuo, Z., Lv, J., Spradling, A.C., and Bellen, H.J. (2015). A genetic toolkit for tagging intronic MiMIC containing genes. Elife *4*, e08469.
49. Nagarkar-Jaiswal, S., Lee, P.T., Campbell, M.E., Chen, K., Anguiano-Zarate, S., Gutierrez, M.C., Busby, T., Lin, W.W., He, Y., Schulze, K.L., et al. (2015). A library of MiMICs allows tagging of genes and reversible, spatial and temporal knockdown of proteins in Drosophila. Elife *4*, e05338.
50. Parks, A.L., Cook, K.R., Belvin, M., Dompe, N.A., Fawcett, R., Huppert, K., Tan, L.R., Winter, C.G., Bogart, K.P., Deal, J.E., et al. (2004). Systematic generation of high-resolution deletion coverage of the Drosophila melanogaster genome. Nat. Genet. *36*, 288–292.
51. Lu, S., Ma, M., Mao, X., Bacino, C.A., Jankovic, J., Sutton, V.R., Bartley, J.A., Wang, X., Rosenfeld, J.A., Beleza-Meireles, A., et al. (2022). De novo variants in FRMD5 are associated with developmental delay, intellectual disability, ataxia, and abnormalities of eye movement. Am. J. Hum. Genet. *109*, 1932–1943.
52. Matsumoto, K., Toh-e, A., and Oshima, Y. (1978). Genetic control of galactokinase synthesis in Saccharomyces cerevisiae: evidence for constitutive expression of the positive regulatory gene gal4. J. Bacteriol. *134*, 446–457.
53. McGuire, S.E., Le, P.T., Osborn, A.J., Matsumoto, K., and Davis, R.L. (2003). Spatiotemporal rescue of memory dysfunction in Drosophila. Science *302*, 1765–1768.
54. McGuire, S.E., Mao, Z., and Davis, R.L. (2004). Spatiotemporal gene expression targeting with the TARGET and gene-switch systems in Drosophila. Sci. STKE *2004*, pl6.
55. Pfeiffenberger, C., Lear, B.C., Keegan, K.P., and Allada, R. (2010). Locomotor activity level monitoring using the Drosophila Activity Monitoring (DAM) System. Cold Spring Harb. Protoc. *2010*. pdb.prot5518.
56. Hata, T., and Nakayama, M. (2007). Targeted disruption of the murine large nuclear KIAA1440/Ints1 protein causes growth arrest in early blastocyst stage embryos and eventual apoptotic cell death. Biochim. Biophys. Acta *1773*, 1039–1051.

57. Rutkowski, R.J., and Warren, W.D. (2009). Phenotypic analysis of deflated/Ints7 function in Drosophila development. Dev. Dyn. *238*, 1131–1139.
58. Tao, S., Cai, Y., and Sampath, K. (2009). The Integrator subunits function in hematopoiesis by modulating Smad/BMP signaling. Development *136*, 2757–2765.
59. Kapp, L.D., Abrams, E.W., Marlow, F.L., and Mullins, M.C. (2013). The integrator complex subunit 6 (Ints6) confines the dorsal organizer in vertebrate embryogenesis. PLoS Genet. *9*, e1003822.
60. Gómez-Orte, E., Sáenz-Narciso, B., Zheleva, A., Ezcurra, B., de Toro, M., López, R., Gastaca, I., Nilsen, H., Sacristán, M.P., Schnabel, R., et al. (2019). Disruption of the Caenorhabditis elegans Integrator complex triggers a non-conventional transcriptional mechanism beyond snRNA genes. PLoS Genet. *15*, 1–31.
61. Zhang, P., Sui, P., Chen, S., Guo, Y., Li, Y., Ge, G., Zhu, G., Yang, H., Rogers, C.M., Sung, P., et al. (2021). INTS11 regulates hematopoiesis by promoting PRC2 function. Sci. Adv. *7*, eabh1684.
62. Cihlarova, Z., Kubovciak, J., Sobol, M., Krejcikova, K., Sachova, J., Kolar, M., Stanek, D., Barinka, C., Yoon, G., Caldecott, K.W., and Hanzlikova, H. (2022). BRAT1 links Integrator and defective RNA processing with neurodegeneration. Nat. Commun. *13*, 5026–5114.
63. Li, W., Wu, S., Xu, H., Zhao, X., Pan, Y., Huang, H., Lv, H., Zhu, X., and Liu, Y. (2022). Novel variant in BRAT1 with the lethal neonatal rigidity and multifocal seizure syndrome. Pediatr. Res. *91*, 565–571.
64. Puffenberger, E.G., Jinks, R.N., Sougnez, C., Cibulskis, K., Willert, R.A., Achilly, N.P., Cassidy, R.P., Fiorentini, C.J., Heiken, K.F., Lawrence, J.J., et al. (2012). Genetic mapping and exome sequencing identify variants associated with five novel diseases. PLoS One *7*, e28936.
65. Straussberg, R., Ganelin-Cohen, E., Goldberg-Stern, H., Tzur, S., Behar, D.M., Smirin-Yosef, P., Salmon-Divon, M., and Basel-Vanagaite, L. (2015). Lethal neonatal rigidity and multifocal seizure syndrome - Report of another family with a BRAT1 mutation. Eur. J. Paediatr. Neurol. *19*, 240–242.
66. Hanes, I., Kozenko, M., and Callen, D.J.A. (2015). Lethal neonatal rigidity and multifocal seizure syndrome–a misnamed disorder? Pediatr. Neurol. *53*, 535–540.
67. Mundy, S.A., Krock, B.L., Mao, R., and Shen, J.J. (2016). BRAT1-related disease–identification of a patient without early lethality. Am. J. Med. Genet. *170*, 699–702.
68. Scheffer, I.E., Boysen, K.E., Schneider, A.L., Myers, C.T., Mehaffey, M.G., Rochtus, A.M., Yuen, Y.P., Ronen, G.M., Chak, W.K., Gill, D., et al. (2020). BRAT1 encephalopathy: a recessive cause of epilepsy of infancy with migrating focal seizures. Dev. Med. Child Neurol. *62*, 1096–1099.
69. Valence, S., Cochet, E., Rougeot, C., Garel, C., Chantot-Bastaraud, S., Lainey, E., Afenjar, A., Barthez, M.A., Bednarek, N., Doummar, D., et al. (2019). Exome sequencing in congenital ataxia identifies two new candidate genes and highlights a pathophysiological link between some congenital ataxias and early infantile epileptic encephalopathies. Genet. Med. *21*, 553–563.
70. Nuovo, S., Baglioni, V., De Mori, R., Tardivo, S., Caputi, C., Ginevrino, M., Micalizzi, A., Masuelli, L., Federici, G., Casella, A., et al. (2022). Clinical variability at the mild end of BRAT1-related spectrum: Evidence from two families with genotype-phenotype discordance. Hum. Mutat. *43*, 67–73.
71. Mahjoub, A., Cihlarova, Z., Tétreault, M., MacNeil, L., Sondheimer, N., Caldecott, K.W., Hanzlikova, H., Yoon, G.; and Care4Rare Canada Consortium (2019). Homozygous pathogenic variant in BRAT1 associated with nonprogressive cerebellar ataxia. Neurol. Genet. *5*, e359.