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Supplemental information

A syndromic neurodevelopmental disorder caused by rare variants in *PPFIA3*

Maimuna S. Paul, Sydney L. Michener, Hongling Pan, Hiuling Chan, Jessica M. Pfliger, Jill A. Rosenfeld, Vanesa C. Lerma, Alyssa Tran, Megan A. Longley, Richard A. Lewis, Monika Weisz-Hubshman, Mir Reza Bekheirnia, Nasim Bekheirnia, Lauren Massingham, Michael Zech, Matias Wagner, Hartmut Engels, Kirsten Cremer, Elisabeth Mangold, Sophia Peters, Jessica Trautmann, Jessica L. Mester, Maria J. Guillen Sacoto, Richard Person, Pamela P. McDonnell, Stacey R. Cohen, Laina Lusk, Ana S.A. Cohen, Jean-Baptiste Le Pichon, Tomi Pastinen, Dihong Zhou, Kendra Engleman, Caroline Racine, Laurence Faivre, Sébastien Moutton, Anne-Sophie Denommé-Pichon, Hyun Yong Koh, Annapurna Poduri, Jeffrey Bolton, Cordula Knopp, Dong Sun Julia Suh, Andrea Maier, Mehran Beiraghi Toosi, Ehsan Ghayoor Karimiani, Reza Maroofian, Gerald Bradley Schaefer, Vijayalakshmi Ramakumaran, Pradeep Vasudevan, Chitra Prasad, Matthew Osmond, Sarah Schuhmann, Georgia Vasileiou, Sophie Russ-Hall, Ingrid E. Scheffer, Gemma L. Carvill, Heather Mefford, Undiagnosed Diseases Network, Carlos A. Bacino, Brendan H. Lee, and Hsiao-Tuan Chao

SUPPLEMENTAL NOTE: CASE REPORTS

Individual 1 - *de novo PPFIA3* NM_003660.4: c.115 C>T (p.Arg39Cys), GRCh37 (hg19)

Individual 1 is a 16-year-old left-handed white male with global delayed development, intellectual disability, epilepsy, strabismus, hypotonia, hyperreflexia, short stature, self-injurious behaviors, aggression, and dysmorphisms.

He was born at 33-weeks gestational age to a 36-year-old mother and a 29-year-old father. Birth length: 43 cm; birth weight: 1.92 kg. His first year of life was notable for feeding difficulties and delayed development. He held his head up when prone at two months, rolled back to front at one year, sat without support at 11-months old, and babbled at 12-months old. Between three to four-years old, he achieved walking and running independently and had one word other than mama or dada. By four-years old, he can scribble. By five-years old, he follows one-step commands and knows body parts. Social development is notable for good eye contact, lack of stranger anxiety, playing in parallel with others at 10-years old, and playing cooperatively with others at 15-years old.

He had epilepsy onset at two-years old with EEG findings notable for focal seizures localizing to the right frontal area and seizure semiology consistent with right frontal onset with a version of the head to the left and jerking of the left arm with secondary generalization.

At 16-years of age, his clinical exam was significant for dysmorphisms including prominent forehead, flared eyebrows with long eyelashes, low-set ears, broad nasal bridge, long columella, thin upper lip, macrodontia, wide-spaced teeth, and wide square feet. He has anxiety, ADHD, and congenital esotropia. His head circumference at 16-years old is 54 cm, which is the 7%tile. His current weight is 45.5 kg. MRI brain at 16-years old was unremarkable with no neuroanatomical seizure locus identified. Genetic testing with trio whole exome sequencing identified a *de novo PPFIA3* c.115C>T (p.Arg39Cys) variant.

Individual 2 - *de novo PPFIA3* NM_003660.4: c.115 C>T (p.Arg39Cys), GRCh37 (hg19)

Individual 2 is a 13-year-old male with global delayed development, intellectual disability, epilepsy, strabismus, right cryptorchidism with orchiopexy at 27 months old, wide-based gait, dysarthric speech, dysmorphisms, and abnormal MRI brain findings.

He was born at 33-weeks gestational age to a 36-year-old mother. Birth weight was 1.99 kg. Pregnancy history was complicated by maternal gestational diabetes requiring insulin, hypothyroidism requiring thyroid medication, and frequency urinary tract infections treated with Macrobid. Treatment involved 16 weeks of progesterone shots for a history of previous preterm pregnancy. APGARs were 4 (at 1 minute), 8 (at 5 minute), and 7 (at 10 minute). Intubated for respiratory distress within one day of life in the setting of ventilator and supplemental oxygen therapy.

His first year of life was notable for delayed developmental milestones. He held his head up, babbled, sat without support, and rolled front to back by his first year of life. He started to crawl at 17-months and could pull to stand and walk independently by 30-months. By the age of four-years old, he ran, climbed the stairs, scribbled, unbuttoned, and undressed, laughed, used mama and dada specifically, followed one-step commands, played in parallel with others,

and played cooperatively with others. However, he was slightly difficult to follow two-step commands and had reduced eye contact.

His first epileptic seizure occurred at two-years of age and his EEG findings were notable for focal seizures localizing to the left temporal area and generalized interictal spikes. At the age 13-years old, his clinical exam was significant for limited speech abilities limited to one-word phrases and dysmorphism including plagiocephaly, bilateral clubfoot, and dysmorphic features. He has anxiety and ADHD. Concerns were raised about his growth rate, with his height of 140 cm (Z score -2.74), weight of 33.9 kg (Z score -2.34), and BMI of 17.3 kg/m^2. His MRI brain findings were normal but there were a few patchy areas of T2 prolongation in the right periatrial white matter. Genetic testing in the setting of trio whole exome sequencing identified a *de novo PPFIA3* c.115 C>T (p.Arg39Cys) variant.

Individual 3 - Proband *PPFIA3* NM_003660.4: c.118 G>A (p.Glu40Lys), GRCh37 (hg19)

Individual 3 is a 22-year-old right-handed white female with global developmental delay, spastic diplegic cerebral palsy, epilepsy, ataxic gait, dysarthria, urinary incontinence, scoliosis and facial dysmorphism. She also presented with anterior pituitary insufficiency (most likely of ischemic origin) with central hypothyroidism, growth hormone deficiency with short stature, secondary adrenocortical insufficiency, and hypogonadotropic hypogonadism with pubertas tarda (delayed puberty). Furthermore, she has severe gastroesophageal reflux disease with esophageal stenosis, hypochromic microcytic anemia, and multiple tooth extractions.

She was born at 32-weeks gestational age to a 37-year-old mother and a 37-year-old father. Birth length: 35 cm (<1%tile, Z score -2.65); birth weight: 1.315 kg (8%tile, Z score -1.43). At the age of three months the patient had severe pertussis infection requiring reanimation and ventilation. Her first year of life was notable for global delayed development and being able to laugh. By three years old she was able to roll front to back, roll back to front, pull to stand, and babble. By six-years old she was able to use mama and dada specifically, use one word other than mama and dada, sit without support, and scribble. At the last examination at 22-years old she was able to undress, wave bye, understand no, follow one and two-step commands, know body parts, smile socially, play in parallel with others, play cooperatively with others, and make eye contact. She was able to walk and to climb stairs with support.

She had epilepsy onset in childhood with an abnormal EEG notable for absence seizures, repeated generalized spike-slow-waves, polyspikes, and sharp waves, bilateral frontal to occipital. At 10-years old, MRI findings demonstrated mild cerebral volume loss. At 22-years old, the clinical exam was significant by spasticity, gait ataxia, dysarthria and dysmorphisms including hypotonic facies, wide mouth, prominent lips, and macroglossia. At 22-years old, her head circumference was 56 cm (73%tile), height 137 cm (<1%tile, Z score -4.86), and weight 47 kg (BMI: 25 kg/m^2). Proband genetic testing with whole exome sequencing identified a *PPFIA3* c.118 G>A (p.Glu40Lys) variant. The variant was not present in the mother. A blood sample of the father for further examination was not available.

Individual 4 – Proband *PPFIA3* NM_003660.4: c.239 A>C (p.Gln80Pro), GRCh37 (hg19)

Individual 4 is a 22-month-old white female with global developmental delays, dysarthria, autistic features, hypotonia, astigmatism, constipation, and dysmorphisms.

She was born at 38-weeks gestational age to a 19-year-old mother. Birth length: 48.26 cm; Birth weight: 3.4 kg. Her biological mother has cognitive delays, functions at a 12-year-old level, and lives in a residential facility. Maternal grandmother and aunt have epilepsy and biological father has suspected autism. Her first year of life was notable for developmental delay and the ability to sit independently. At 13-months old she was "wobbly" when sitting but was able to pick up toys and transfer objects from one hand to another, crawling, and cruising. She was not walking at 13-months old. She was nonverbal but communicated through vocalizations and was able to smile and laugh. At 22-months old she could take a few steps alone, play peek a boo and can mimic hand gestures. She can communicate by grabbing mom's arm. She can hold her own food pouches and pick up small items.

At 22-months old, her clinical exam is significant by dysmorphisms including bitemporal narrowing, upslanting eyebrows, upslanting palpebral fissures, telecanthus, epicanthal folds, depressed nasal bridge, and anteverted nares. She has suspected autism, but it is unable to be assessed adequately at initial developmental evaluation due to age and cognitive delay. Brain MRI findings are normal. Her head circumference at 22-months old is 47 cm which is 53rd percentile. At latest assessment her height was 85.8 cm, weight was 12.3 kg, and BMI was 16.7 kg/m^2. Proband genetic testing with whole exome sequencing identified a *PPFIA3* c.239 A>C (p.Gln80Pro) variant of unknown inheritance.

Individual 5 – Maternally inherited *PPFIA3* NM_003660.4: c.240+1 G>A, GRCh37 (hg19)

Individual 5 is a five-year-old white female with global delayed development, intellectual disability, hyperreflexia, microcephaly (-3 SD), strabismus, and facial features.

She was born at 38-weeks gestational age to a 25-year-old mother and a 25-year-old father. Birth length: 47 cm; birth weight: 2.85 kg. Head circumference at birth was 31 cm, which is in the 2% tile. Both parents present with intellectual disability. Family history is notable for autism spectrum disorder, intellectual disability, global delayed development, and microcephaly. Her first year of life was notable for global delayed development and sitting independently. By 19-months old, she was able to walk independently.

At 5-years old, her clinical exam is significant for speech, gross, and fine motor delay and dysmorphism. She has ADHD. Her head circumference at five years of age is 46 cm, which is the 1% tile. Height at latest assessment: 98.2 cm; weight at latest assessment: 13 kg; BMI: 13.5 kg/m^2. MRI was unremarkable. Genetic testing with exome sequencing identified a maternally inherited *PPFIA3* c.240+1 G>A variant. Mother is individual 6.

Individual 6 – Proband *PPFIA3* NM_003660.4: c.240+1 G>A, GRCh37 (hg19)

Individual 6 is a 35-year-old white female with microcephaly (-2 SD), hyperthyroidism, a history of miscarriages, and intellectual disability. Family history is notable for autism spectrum disorder and intellectual disability. Height at latest assessment was160 cm; weight at latest assessment was 76 kg (BMI: 29.7 kg/m^2). Proband genetic testing with exome sequencing identified a *PPFIA3* c.240+1 G>A variant of unknown inheritance.

Individual 7- *de novo PPFIA3* NM_003660.4: c.943 G>T (p.Ala315Ser), GRCh37 (hg19)

Individual 7 was a Latino female deceased neonatally with genitourinary malformations, microcephaly, hypoglycemia, hydronephrosis, renal failure, newborn sepsis, feeding difficulties,

and dysmorphisms including anorectal malformations, absent bladder, bilateral cystic renal dysplasia, cloacal anomaly, anal atresia, and ambiguous genitalia. Born at 33-weeks gestation to a 32-year-old mother. Mother was diabetic. Birth length: 46.9 cm; Birth weight: 2.44 kg. Head circumference at birth was 31.2 cm, which is the 1%tile. Genetic testing with trio whole exome sequencing identified a *de novo PPFIA3* c.943 G>T (p.Ala315Ser) variant.

Individual 8 - *de novo PPFIA3* NM_003660.4: c.1243 C>T (p.Arg415Trp), GRCh37 (hg19)

Individual 8 is an eight-year-old ambidextrous, mixed European and Asian female with severe global delayed development and intellectual disability, macrocephaly, medically refractory epilepsy, dysmorphisms, dysarthria, dysphagia, congenital hypotonia, gastroesophageal reflux disease, constipation, motor stereotypies, mild tremor, infantile exotropia, delayed visual maturation, persistent sialorrhea, hydronephrosis, recurrent UTI's, and frequent pneumonia.

She was born at 40-weeks gestational age to a 31-year-old mother and a 36-year-old father. Birth length: 53.5 cm; Birth weight: 3.86 kg. Head circumference at birth was 39 cm, which is the 96%tile. Her first year of life was notable for feeding difficulties and global delayed development. By 10 months of age, she was able to hold her head up when prone, roll front to back, roll back to front, coo, laugh socially, and smile socially. At 18 months of age, she was able to sit without support. By three years of age, she was able to pull to stand, cruise, and wave bye-bye. At seven years of age, she was able to walk independently with a stiff gait. At eight years of age, she can scribble, transfer objects between hands, bring hands to midline, make eye contact, and play in parallel with others.

She had epilepsy onset at two to three years of age with EEG findings (at 5 years of age) notable for frequent bifrontal and right frontal temporal region spike-wave discharges. MRI findings at four months of age indicated flattening of the posterior globes at the level of the insertion of the optic nerves. MRI findings at five years old were unremarkable.

At eight years of age, clinical assessment was significant for autistic features and dysmorphisms including a broad prominent forehead, broad nose, wide mouth with protruding lower lip and prominent gums, widely spaced teeth, and broad toes. Her head circumference is 54.5 cm which is the 99%tile. Height at latest assessment: 119 cm (6%tile); weight at latest assessment: 22.7 kg (20%tile); BMI: 16 kg/m^2 (56%tile). Genetic testing with trio whole exome sequencing identified a *de novo PPFIA3* c.1243 C>T (p.Arg415Trp) variant.

Individual 9 – Proband NM_003660.4: c.1243 C>T (p.Arg415Trp), GRCh37 (hg19)

Individual 9 is a 10-year-old female of Middle Eastern descent with intellectual disability, global delayed development, epilepsy, hypomimia, weak/absent cry, autistic features, nonverbal, nonambulatory, hypertonia, incontinence, strabismus, gastroesophageal reflux disease, and dysmorphisms.

Head circumference at birth was 34 cm, which is the 29%tile. Birth length: 49 cm; birth weight: 22 kg. Her first year of life was significant for global delayed development and feeding difficulties.

Abnormal EEG findings were significant for bilateral epileptiform discharges. At 10-years old her clinical exam was notable for autistic features and dysmorphisms including prominent

forehead, dysmorphic facies, class 2 malocclusion, tall forehead, long, triangular face, clinodactyly, short stature, and pointed chin. MRI brain findings at 10-years old were unremarkable. Head circumference was 49.5 cm, which is the 1%tile. Height at latest assessment: 125 cm; weight at latest assessment: 18.5 kg (BMI: 11.8 kg/m^2). Genetic testing with proband whole exome sequencing identified a *PPFIA3* c.1243 C>T (p.Arg415Trp) variant of unknown inheritance.

Individual 10 – *de novo PPFIA3* NM_003660.4: c.1285 C>T (p.Arg429Trp), GRCh37 (hg19)

Individual 10 was a white female fetus from an elective pregnancy termination in the 27th week of gestation due to abnormal brain gyration and ventriculomegaly with a heart defect (thin aorta, left ventricle larger than right ventricle) identified through prenatal MRI and ultrasound. Pregnancy was the result of intracytoplasmic sperm injection (ICSI). Pathological examination could confirm MRI and ultrasound findings and additionally revealed an esophageal atresia with esophago-tracheal fistula (Vogt IIIb). Genetic testing with trio whole exome sequencing of fetal skin identified a *de novo PPFIA3* c.1285 C>T (p.Arg429Trp) variant in 26% of the reads.

Individual 11 – *de novo PPFIA3* NM_003660.4: c.1492 C>T (p.Arg498Trp), GRCh37 (hg19)

Individual 11 is a six-year-old male originating from Syria with global delayed development, intellectual disability, autistic features, and mild dysmorphism.

He was born to a 22-year-old mother and a 38-year-old father after an uneventful pregnancy with a birth length of 51 cm and a birth weight of 3.5 kg. His motor development was normal with walking independently at 11-months old and later able to run, climb, swim and ride a bike. He spoke first words within his first year of life but showed delayed further speech development. He is now able to speak in easy sentences with incorrect grammar in Arabic and German. Developmental assessment revealed mild intellectual disability with an IQ of 65. At the age of four-years old, autism was diagnosed because of poor eye contact and poor social interaction. Autistic features improved over time, but the individual is hyperactive and shows reduced concentration ability. The individual attends a special needs school. He did not show organic malformations, nor did he develop seizures.

The individual has a brother with autism and NDD harboring a *de novo* variant in *HNRNPD* (no further information is available). Two further siblings are healthy.

His current clinical examinations at six-years old are notable for delayed speech, ADHD, and mild dysmorphism including synophrys, almond-shaped eyes, slightly down slanting palpebral fissures, bulbous nasal tip and large, deeply set ears. Current speech ability is made up of short sentences in Arabic and German, with grammar and pronunciation mistakes. He was friendly and cooperative during the examination. He presented with a head circumference of 52 cm (34. percentile) a height of 134 cm (97%tile) and a weight of 33.8 kg (97%tile, BMI: 18.8 kg/m^2). Genetic testing with trio whole exome sequencing identified a *de novo PPFIA3* c.1492 C>T (p.Arg498Trp) variant.

Individual 12 – *de novo PPFIA3* NM_003660.4: c.1638 G>T (p.Trp546Cys), GRCh37 (hg19)

Individual 12 is an 11-year-old white male with global delayed development, intellectual disability, autism spectrum disorder, motor stereotypies, PANDAS (Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal Infections), and hematuria.

He was born to term to a 31-year-old mother and a 31-year-old father. Birth length: 52.07 cm; birth weight: 3.28 kg. He could sit independently at six-months old, stand without support at seven-months old, and walk independently at 11-months old. MRI brain at two-years old was unremarkable.

At six-years old, his clinical exam was significant for autistic-like speech patterns, and slow weight gain. Height at latest assessment: 143.5 cm; weight at latest assessment: 27.67 kg (BMI: 13.5 kg/m^2). Genetic testing with trio whole exome sequencing identified a *de novo PPFIA3* c.1638 G>T (p.Trp546Cys) variant.

Individual 13 – *de novo PPFIA3* NM_003660.4: c.2350 C>T (p.Arg784Trp), GRCh37 (hg19)

Individual 13 is a 16-year-old white female with intellectual disability, epilepsy, Landau Kleffner syndrome mild speech, constipation, thoracolumbar scoliosis and social delay.

She was born to a 33-year-old mother and a 36-year-old father. Family history is notable for a mother with depression, a half-brother with psychiatric problems and substance abuse, and a brother who died at five months from aortic valve stenosis. She was able to walk independently at 12-months old. At five-years old, she had mild delay in speech, motor, and social functions. At six-years old, she had speech regressions with auditory-verbal agnosia, short, difficult-to-understand sentences, and expressive and receptive aphasia. At seven-years old, expressive and receptive language skills were improved, had difficulty with poly-syllabic words, verbal skills below nonverbal skills, and was learning sign language. At eight-years old, her language was severely impaired with age-appropriate nonverbal functioning. At 11-years old, she had primary enuresis. At 13-years old, FSIQ 69, borderline nonverbal cognition, intellectually deficient verbal skills, receptive language skills level was at four years and 7-months old.

Epilepsy onset at six-years old with EEG findings notable for frequent focal discharges with perisylvian dipole over one hemisphere. MRI brain at six-years old was unremarkable with no neuroanatomical seizure locus identified. EEG findings at seven years old were significant for continuous spike-wave in slow-wave sleep.

At 16-years old, her clinical examination is significant for mild intellectual disability, epilepsy, abnormal EEG, cow's milk intolerance, and mild speech delay. She also has depression, mood disorder, behavioral problems, substance abuse, and post-traumatic stress disorder (PTSD). Genetic testing with trio whole exome sequencing identified a *de novo PPFIA3* c.2350 C>T (p.Arg784Trp) variant seen in gnomAD (v2.1.1) at a frequency of 3.19x10-5 (1/31,386).

Individual 14 – *de novo* NM_003660.4: c.2609 T>A (p.Ile870Asn), GRCh37 (hg19)

Individual 14 is a five-year-old white female with speech delay, social delay, microcephaly, nonverbal, autism and mixed receptive-expressive language disorder, constipation, and anxiety.

She was born at 36-weeks gestational age to a 34-year-old mother and a 33-year-old father. Twin pregnancy was complicated by twin-twin transfusion syndrome. She is a monozygotic twin with individual 15. Birth weight: 1.18 kg. Family history is significant for the mother with migraine headaches with aura, and proximal weakness due to genetic muscular

dystrophy, the maternal uncle has same muscular dystrophy and the older brother has dyslexia, dyscalculia, and dysgraphia.

At 11-months old, she was able to roll front to back, roll back to front, sit without support, and smile socially. By 18-months old, she was able to babble and walk independently. At 20-months old, she was able to use mama and dada specifically.

At five-years old, her clinical assessment was significant for severe receptive and expressive language delay, articulation speech disorder, and microcephaly. Her head circumference is 45.2 cm, which is the 1%tile. Height at latest assessment: 101.7 cm; weight at latest assessment: 13.7 kg; BMI: 13.2 kg/m^2. She uses a communication device for 2-3-word phrases. Genetic testing with trio whole exome sequencing identified a *de novo PPFIA3* NM_003660.4: c.2609 T>A (p.Ile870Asn) variant.

Individual 15 - *de novo* NM_003660.4: c.2609 T>A (p.Ile870Asn), GRCh37 (hg19)

Individual 15 is a five-year-old white female with gross motor delay, speech delay, speech apraxia, hypotonia, receptive-expressive language disorder, plagiocephaly, microcephaly, torticollis, constipation, migraines, motor apraxia, and difficulty with depth perception.

She was born at 36 weeks to a 34-year-old mother and a 33-year-old father. Twin pregnancy was complicated by twin-twin transfusion syndrome. She is a monozygotic twin with individual 14. Birth weight: 2.268 kg. Family history is significant for the mother with migraine headaches with aura, and proximal weakness due to genetic muscular dystrophy, the maternal uncle has same muscular dystrophy and the older brother has dyslexia, dyscalculia, and dysgraphia.

At 13-months old, she was able to roll front to back, roll back to front, sit without support, smile socially, and babble. At 18-months old she was able to walk independently. MRI brain at 14-months old was notable for significant right posterior plagiocephaly; mild periventricular signal abnormality with associated mild white matter volume loss, suggesting chronic sequelae of mild periventricular leukomalacia.

At five-years old, her clinical examination was significant for microcephaly, mixed receptive-expressive language disorder/apraxia of speech, and suspected ADHD. Head circumference is 44.9 cm, which is <1%tile. Height at latest assessment: 104.7 cm; weight at latest assessment: 16.1 kg; BMI: 14.7 kg/m^2. Genetic testing with trio whole exome sequencing found a *de novo PPFIA3* c.2609 T>A (p.Ile870Asn) variant.

Individual 16 – Proband *PPFIA3* NM_003660.4: c.2706dup (p.Ser903Leufs*86), GRCh37 (hg19)

Individual 16 is a 23-year-old white male with global developmental delay, intellectual disability, hypotonia, autistic features, motor delay, motor incoordination, speech delay, hypermetropic, monocular exotropia, and ADHD. Height at latest assessment: 162.6 cm; weight at latest assessment: 90.7 kg; BMI: 34.3 kg/m^2. Genetic testing with proband whole exome sequencing found a *PPFIA3* c.2706dup (p.Ser903Leufs*8) variant of unknown inheritance.

Individual 17 – *de novo PPFIA3* NM_003660.4: c.2717 C>T (p.Ser906Leu), GRCh37 (hg19)

Individual 17 is a 13-year-old white female with global developmental delay, intellectual disability, speech delay, macrocephaly, abnormal EEG, dysarthria, hypotonia, autism, astigmatism, and dysmorphisms.

She was born at 41-weeks gestational age to a 24-year-old mother and a 27-year-old father. Birth length: 54 cm; birth weight: 3.8 kg. Head circumference at birth was 38 cm, which is the 98%tile. After birth, contractures of the hips and shoulders were noticeable. Family history was significant for a maternal grand cousin with mild intellectual disability. Individual 17 was able to sit without support at 8-months old and walk independently at 18-months old. She spoke her first words at 30-months old and 2-word sentences around 7-years old. Global developmental delay with moderate intellectual disability and hypotonia were documented. MRI brain imaging at 8-years old was unremarkable.

At 13-years old, her clinical exam was significant for limited speech, suspected scoliosis, and abnormal EEG findings including right fronto-central epileptiform discharges. Her current head circumference was 57 cm, which is the 97%tile. Height at latest assessment: 155.5 cm; Weight at latest assessment: 58.4 kg; BMI: 24.2 kg/m^2. In a recontact with the parents when she was 21-years old, the parents reported an autism spectrum disorder diagnosis. Genetic testing with trio whole exome sequencing identified a *de novo PPFIA3* c.2717 C>T (p.Ser906Leu) variant.

Individual 18 – Proband *PPFIA3* NM_003660.4: c.3307del (p.Glu1103Asnfs*8), GRCh37 (hg19)

Individual 18 is a nine-year-old white female with global delayed development, impulsive behavior, delayed speech, abnormal EEG, hypotonia, and dysmorphisms including bilateral epicanthus which was vanished over the years, right ear slightly different from the left ear, and protruding bilateral costal arch.

She was born at 40-weeks gestational age to a 17-year-old mother and a 16-year-old father. Birth length: 49 cm; birth weight: 3.14 kg. Head circumference at birth was 35 cm, which is the 53%tile. Family history was significant for both parents, two maternal aunts, and a maternal grandmother with mild intellectual disability. She was able to walk independently at 24-months old. MRI brain at two-years old was reported normal.

At nine years of age, her clinical assessment was significant for abnormal EEG without seizures. Her current head circumference is 48.5 cm, which is the 5%tile. Height at latest assessment: 113 cm; weight at latest assessment: 19.3 kg; BMI: 15.1 kg/m^2. Genetic testing with proband whole exome sequencing identified a *PPFIA3* c.3307del (p.Glu1103Asn fs*8) variant.

Individual 19 – Maternally Inherited *PPFIA3* NM_003660.4: deletion exons 22-30, GRCh38 (hg38)

Individual 19 is a seven-year-old white male with delayed attainment of developmental milestones, intellectual disability, speech delay, dysarthria, hypotonia, microcephaly, and bilateral clinodactyly.

Family history is significant for a mother with mild intellectual disability.
He was born at 38-weeks gestational age following an unremarkable pregnancy in good condition weighing 2.595 kg. The head circumference at birth was 33 cm, which is the 2.39 percentile.

He walked independently at two-years old. At seven-years old, there was a history of frequent vacant episodes (absence episodes), recurrent dislocation of radial head, joint laxity, and speech delay. His growth measurements showed height at 118 cm; weight at 18.9 kg; BMI: 13.6 kg/m^2 and head size 49 cm (-3SD). His clinical examination was significant for hypotonia and joint laxity and microcephaly. Current speech ability includes a lack of coherence and limited vocabulary. Genetic testing with trio whole genome sequencing through the 100K genomes project of Genomics England identified a maternally inherited *PPFIA3* exons 22-30 deletion. No further information is available for the precise breakpoints of the deletion of exons 22-30.

Individual 20 – Compound Heterozygous *PPFIA3* NM_003660.4: c.[2377C>A]; c.[2276 A>G] (p.Pro793Thr; p.Lys759Arg), GRCh37 (hg19)

Individual 20 is a nine-year-old male with severe global delay in development, intellectual disability, dystonic diplegia, dysarthria, hypotonia, epilepsy, dysmorphisms, microcephaly, autistic features, severe emotional lability, ataxic gait, lower limb hypertonia, strabismus, and exotropia.

He was born at 39-weeks of gestation to a 29-year-old mother and a 30-year-old father. Birth weight: 3.26 kg. At 3-months old his head circumference was 38.5 cm (25th percentile). At his most recent assessment at nine-years old, he can hold his head up when prone, crawl, pull to stand, know body parts, use one word other than mama and dada, follow one and two-step commands, and smile socially. He is not able to walk independently, wears ankle foot orthotics, needs assistance climbing stairs, and is able to sit in a chair but not on the floor. MRI brain imaging at four-years old identified white matter changes related to hypoxic-ischemic changes.

He had epilepsy onset at seven-years old with EEG findings notable for dysrhythmia grade IV, diffuse background slowing, and multiple independent spikes.

At nine years of age, his clinical examination was significant for severe epilepsy, dysarthria, spasticity, diffuse bilateral white matter changes as sequelae from his hypoxic-ischemic encephalopathy (HIE), and dysmorphisms including dysmorphic facies, prominent forehead, long face, prominent tongue and lips, absence of foot arches, flat nasal bridge, myopathic facies, clinodactyly, and widely spaced teeth. His current speech ability is almost non-verbal, he knows a few words, uses sign language at home, and has an assistive device for communication. Height at latest assessment: 152.4 cm; weight at latest assessment: 45 kg; BMI: 19.4 kg/m^2.

Genetic testing with trio whole exome sequencing identified a maternally inherited c.2377C>A (p.Pro793Thr) variant from an unaffected mother, and a paternally inherited c.2276 A>G (p.Lys759Arg) variant from an unaffected father. The maternally inherited p.(Pro793Thr) variant is seen in gnomAD (v2.1.1) at a frequency of 7.61x10-4 (215/282,366). The paternally inherited p.Lys759Arg variant is seen in gnomAD (v2.1.1) at a frequency of 4.77x10-5 (13/282,880).

SUPPLEMENTAL FIGURES

Figure S1: Evolutionary conservation of the affected protein residues between PPFIA3 and orthologs in vertebrate and invertebrate species. Conservation analysis of affected residues from the 17 individuals with missense variants with neurodevelopmental findings are shown in *H. sapiens* (human), *M. musculus* (mouse), *D. melanogaster* (fruit flies), and *C. elegans* (worm) with the affected residue in red. Residues within or adjacent to a functional domain (coiled-coil or SAM domains) are highly conserved across vertebrates and invertebrates. **(A)** Affected residues in individuals 1-4 and 7-10. **(B)** Affected residues in individuals 11-18, and 20.

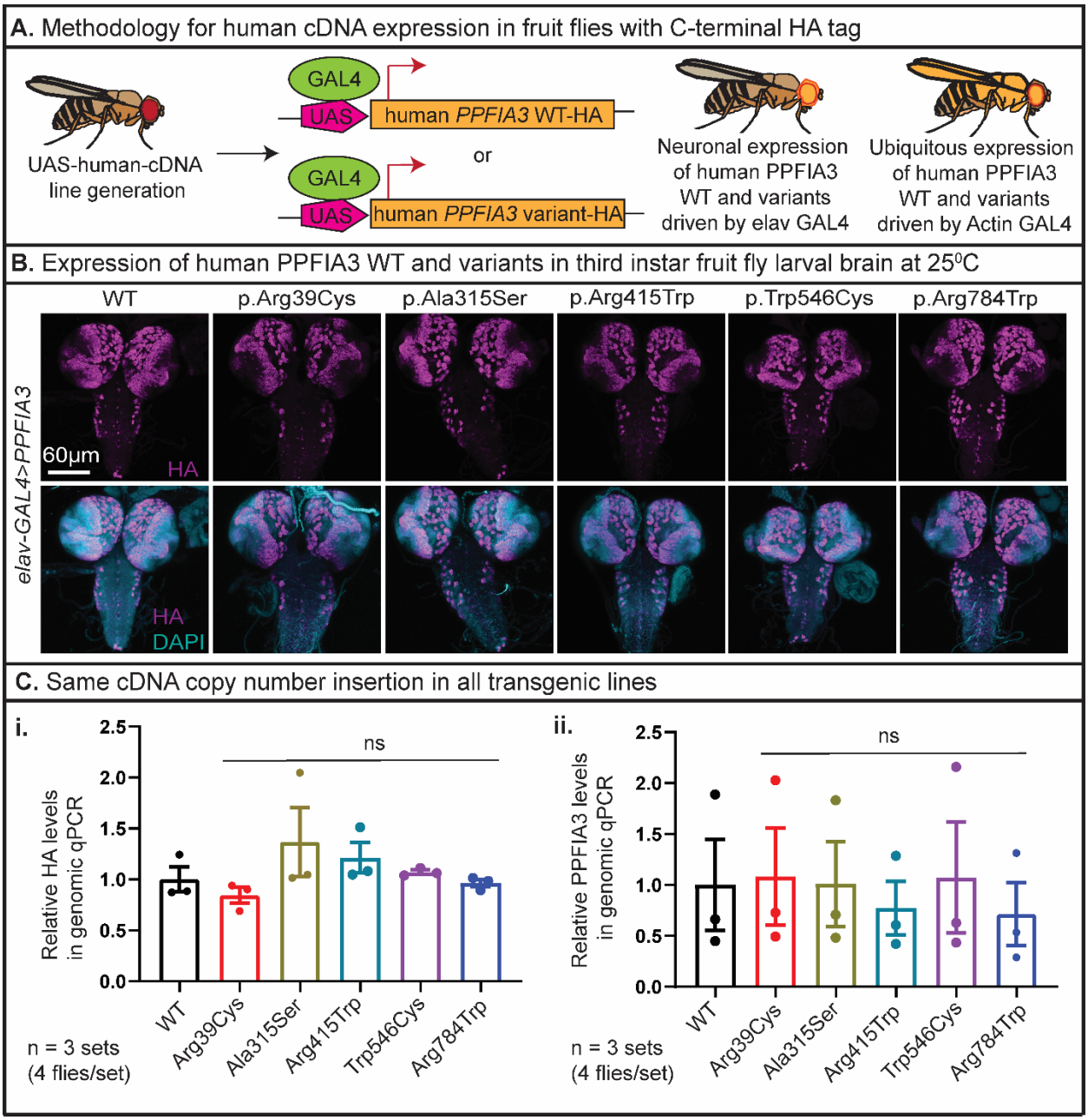


Figure S2: GAL4-UAS targeted expression of PPFIA3 WT and variant cDNAs in fruit flies. **(A)** Methodology for neuron specific and ubiquitous expression of *UAS-PPFIA3*-WT and variant cDNAs using *elav-GAL4* and *Actin-GAL4*, respectively. **(B)** *elav-GAL4* mediated production of PPFIA3 WT and five missense variants are shown in the third instar larval brain using immunostaining against the C-terminal HA epitope tag (magenta). Nuclear staining with DAPI is shown in cyan. Scale bar is 60 µm. Crosses were set and maintained at 25°C. **(C)** qPCR with genomic DNA to detect the expression level of cDNA. No differences in **(i)** HA and **(ii)** PPFIA3 levels were observed between the *UAS-PPFIA3* variants compared to *UAS-PPFIA3* WT. Statistical analysis with one-way ANOVA and Tukey's post-hoc test. Data shown as mean ± standard error of mean (SEM). Non-significance shown as ns.

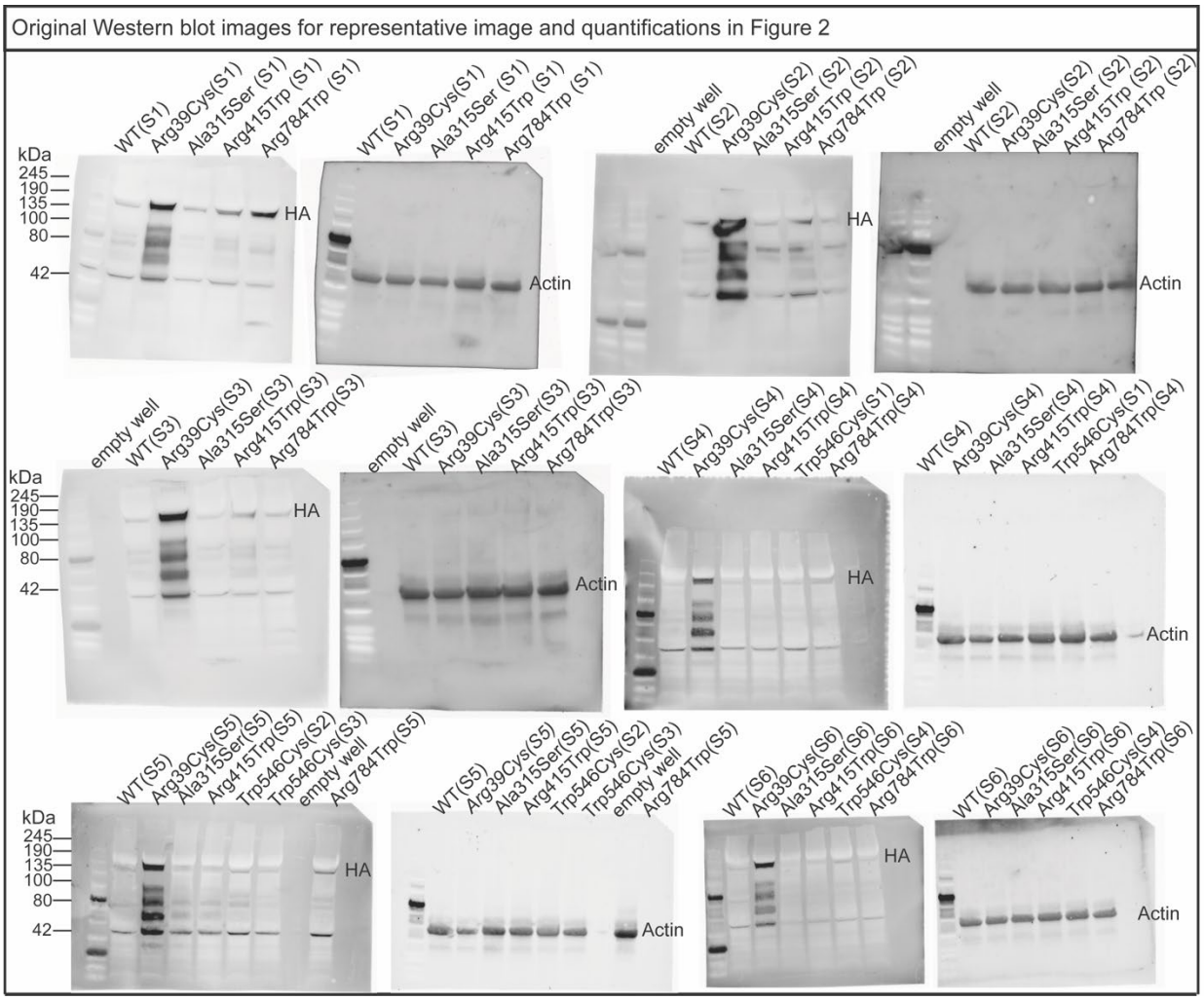


Figure S3: Original Western blot gel images. Raw image of Western blot results used for quantification in Figure 2. Set 1 (S1) through Set 6 (S6) represents the number of biological replicates for each genotype.

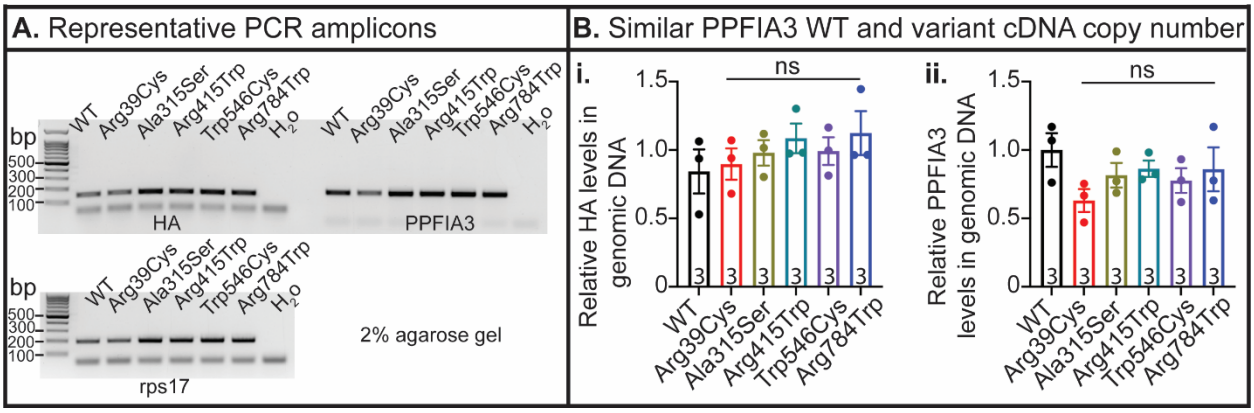


Figure S4: (A) Representative PCR amplicons from genomic DNA showing the relative HA and PPFIA3 levels in *UAS-PPFIA3* WT or variant fly lines relative to the endogenous gene rps17. **(B)** No differences in **(i)** HA and **(ii)** PPFIA3 levels were observed between the *UAS-PPFIA3* variants compared to *UAS-PPFIA3* WT. Statistical analysis with one-way ANOVA and Tukey's post-hoc test. Data shown as mean ± SEM. Non-significance shown as ns.

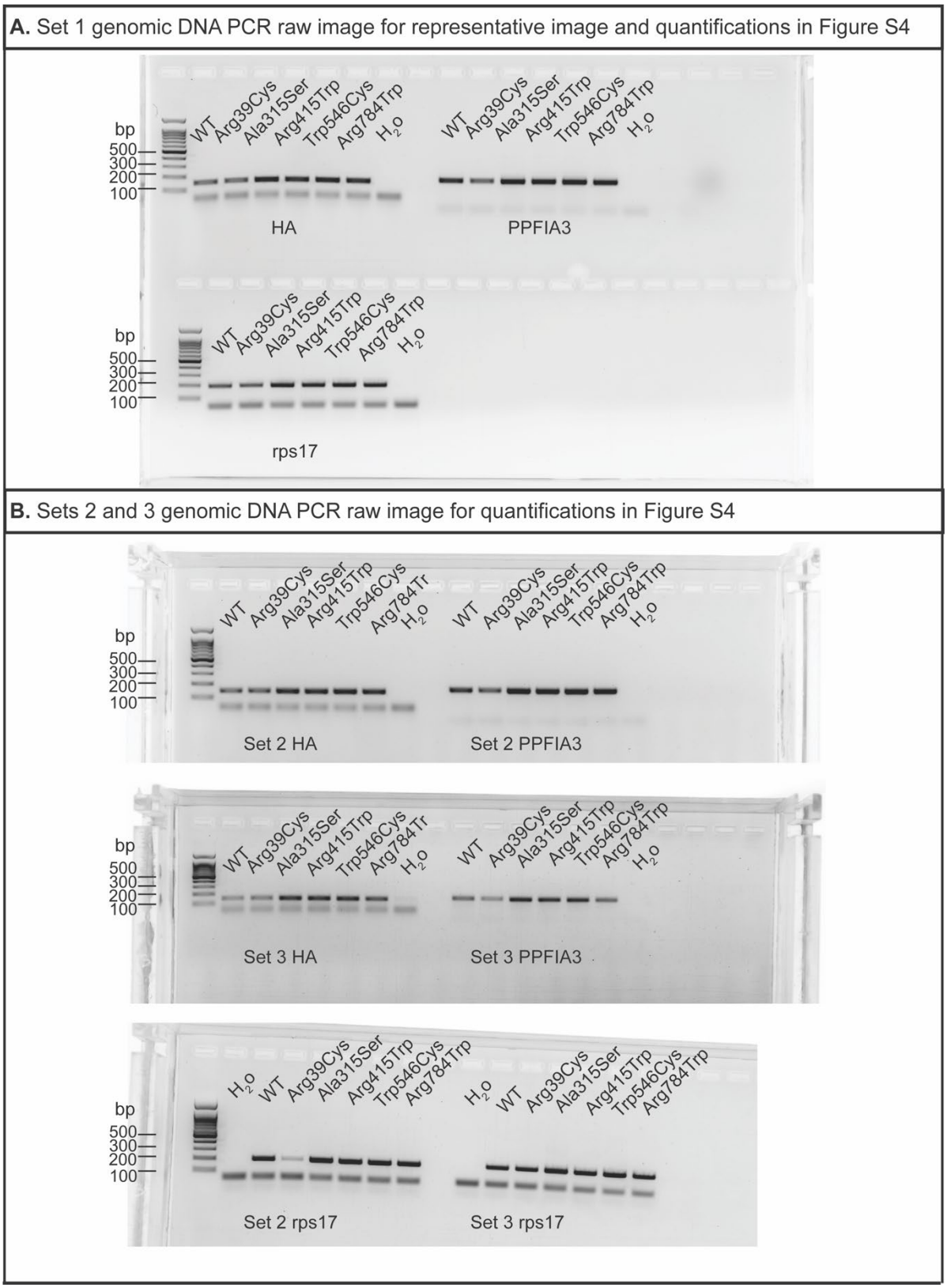


Figure S5: Original genomic PCR DNA gel images. **(A)** Raw image of genomic PCR results from Set 1 used for representative image and quantification in Figure S4. **(B)** Raw image of genomic PCR results from Sets 2 and 3 used for quantification in Figure S4.

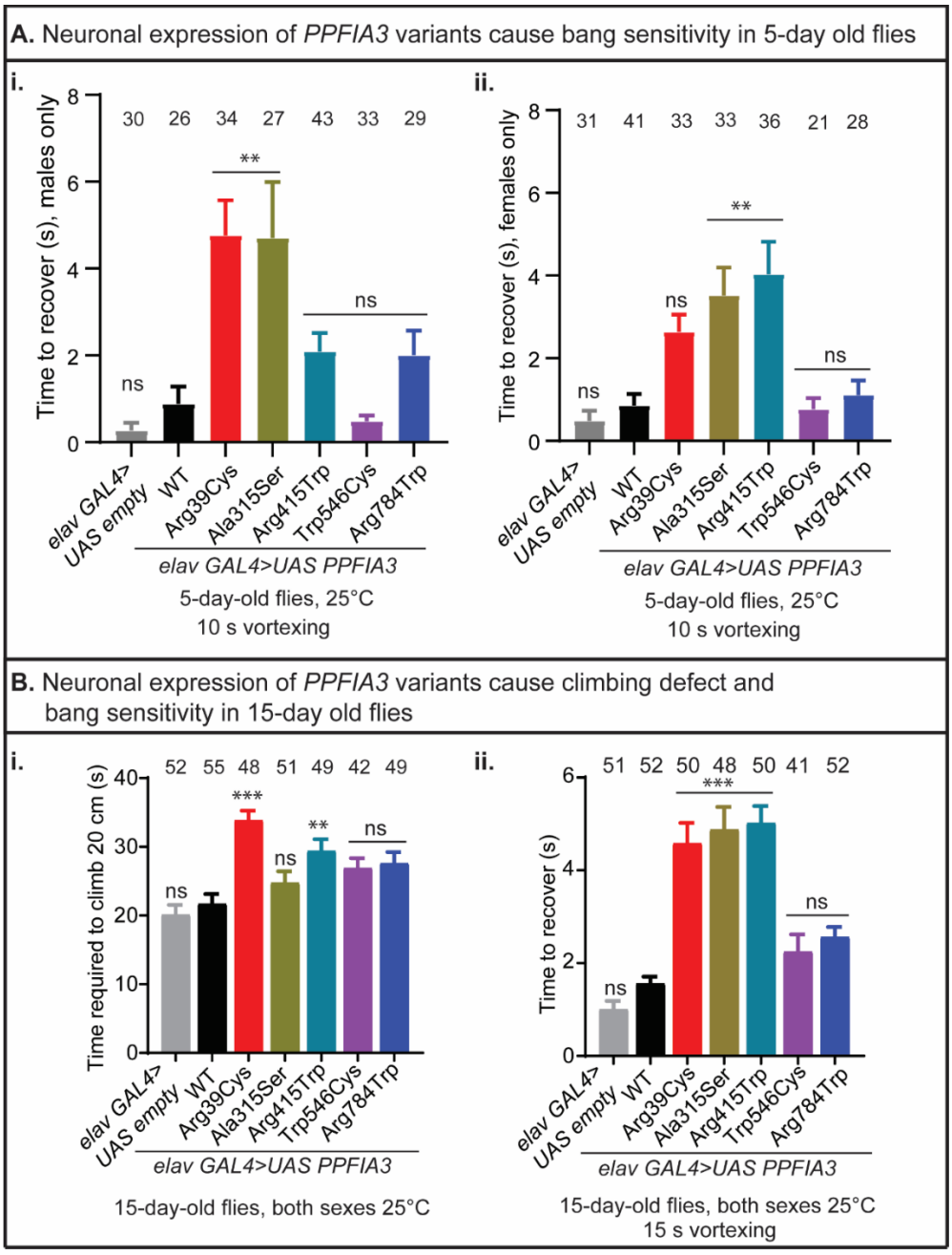


Figure S6: Behavioral assays in fruit flies. **(A)** Bang sensitivity assay with 10s vortexing in 5-day old flies expressing PPFIA3 WT and variants. **(i)** Significant difference in the recovery time was seen p.Arg39Cys- and p.Ala315Ser-expressing males. **(ii)** Significant difference in the recovery time was seen p.Ala315Ser- and p.Arg415Trp-expressing females. **(B) (i)** Climbing assay in 15-day old flies expressing PPFIA3 WT and variants. Significant difference in the time required to climb was seen in p.Arg39Cys- and p.Arg415Trp-expressing flies. **(ii)** Bang sensitivity assay with 15s vortexing in 15-day old flies expressing PPFIA3 WT and variants. Significant difference in the recovery time was seen in p.Arg39Cys-, p.Ala315Ser-, and p.Arg415Trp-expressing flies. Significance shown as $**p<0.01$ and $***p<0.001$. Non-significance shown as ns.

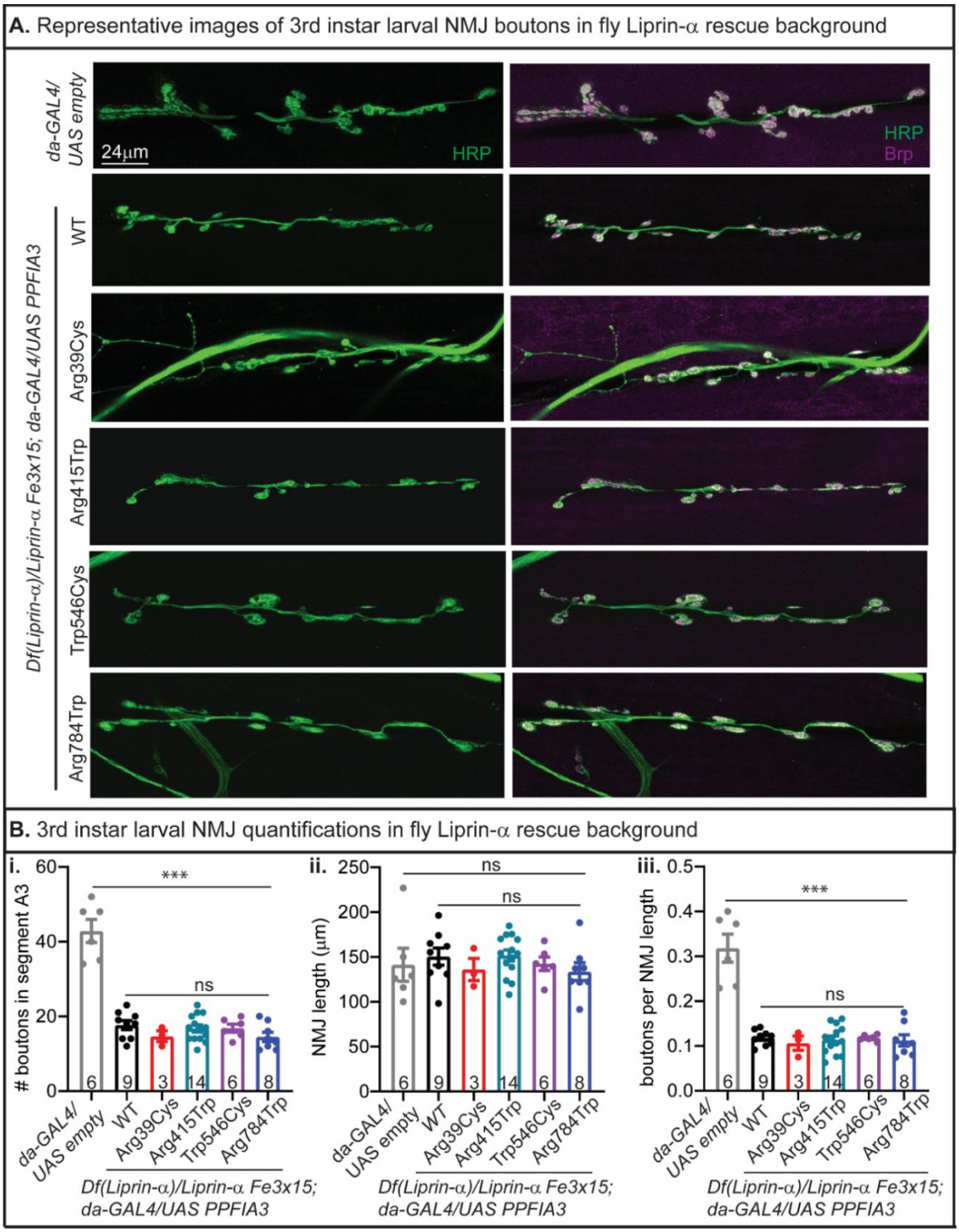
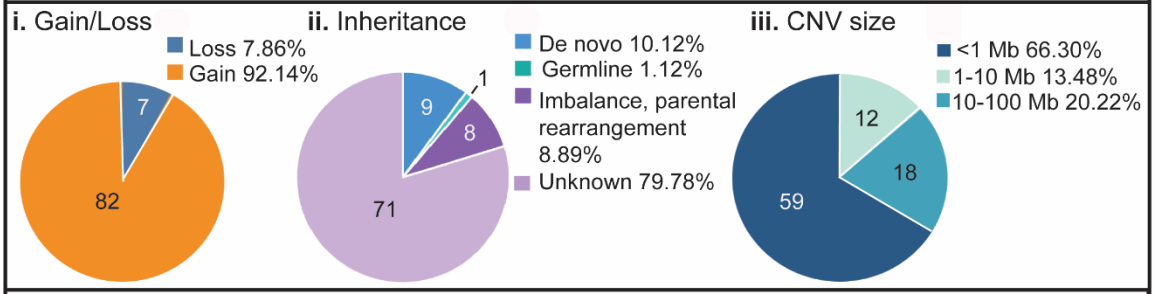


Figure S7: *da-GAL4* mediated ubiquitous expression of *PPFIA3* WT and variant cDNAs in the background of complete loss of Liprin-a result in NMJ bouton loss. **(A)** Representative images of 3rd instar larval NMJs of each genotype including *da-GAL4>UAS* empty, *da GAL4>PPFIA3* WT, p.Arg39Cys, p.Ala315Ser, p.Trp546Cys, and p.Arg784Trp in the complete loss of Liprin-a background is shown. HRP (Horseradish Peroxidase) is a pan-neuronal marker (green) and Brp (Bruchpilot) is an active zone marker (magenta). Scale bar is 24 μm. **(B) (i)** Quantification of

total number of boutons in the muscle 6/7 (abdominal segment A3) NMJ show that the expression of *PPFIA3* WT and variants in the background of *Liprin-a* LOF results in significant loss of boutons compared to the *da-GAL4>UAS* empty control. However, there is no significant difference in the NMJ bouton numbers between *PPFIA3* WT and variants. **(ii)** Quantification of total NMJ length show no significant difference. **(iii)** Quantification of the ratio of boutons per NMJ length show a significance difference between *da-GAL4>UAS* empty control and other genotypes, but there is no significant difference between *PPFIA3* WT and variants. Statistical analysis with one-way ANOVA and Tukey's post-hoc analysis. Data shown as mean ± SEM with the sample size of total number of NMJs shown in figure. Significance shown as ***p<0.001. Non-significance shown as ns.

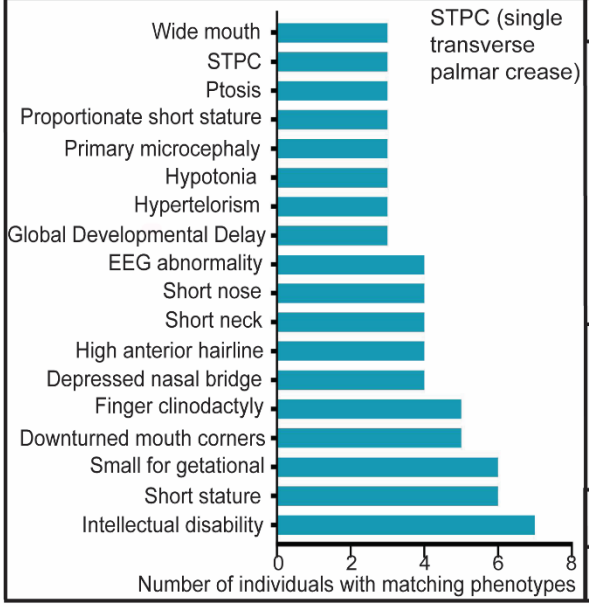
A. CNVs affecting *PPFIA3* in DECIPHER v11.21, gnomAD SVs v2.1, and ClinVar (07/23)



B. CNV losses affecting *PPFIA3* in DECIPHER v11.21, gnomAD SVs v2.1, and ClinVar (07/23)

Source	Sex	Size	Inheritance	Genotype	Coordinates/Reference Genome	Phenotype
Decipher 385478	46XX	417.5 kb	De novo	Het	Chr19:(48782425-49199984)x1 GRCh38	Autistic behavior; Mild microcephaly
Decipher 455208	46XX	23.57 Mb	Unknown	Het	Chr19:(32625762-56194051)x1 GRCh38	N/A
ClinVar 1951732	N/A	242.2 kb	Unknown	N/A	Chr19:(49472545-49714755)x1 GRCh37	Progressive familial heart block type IB
ClinVar 607431	N/A	9.8 kb	Unknown	N/A	Chr19:(49651042-49660889)x1 GRCh37	N/A
ClinVar 607427	N/A	94.8 kb	Unknown	N/A	Ch19: (49556215-49651042)x1 GRCh37	N/A
gnomAD DEL_19_173200	N/A	71 bp	Unknown	N/A	Chr19:(49644129 - 49644200)x1 GRCh37	N/A
gnomAD DEL_19_173200	N/A	71 bp	Unknown	N/A	Chr19:(49644129 - 49644200)x1 GRCh37	N/A

C. *PPFIA3* CNV (>2/24) phenotypes, DECIPHER



D. *PPFIA3* CNV (2/24) phenotypes, DECIPHER

Category	Phenotypes
Dysmorphisms	Aplasia/Hypoplasia of the earlobes; Barrel-shaped chest; Coarse hair; Frontal bossing; Long eyelashes; Long philtrum; Low-set ears; Mandibular prognathia; Microcephaly; Micrognathia; Micropenis;Posteriorly rotated ears; Redundant neck skin; Sacral dimple; Short philtrum; Wide nasal base
Neurology	Delayed speech and language development; Febrile seizure (within the age range of 3 months-6 years); Hydrocephalus; Hypoplasia of the corpus callosum; Strabismus
Cardiology	Tetralogy of Fallot
Genitourinary	Cryptorchidism

Figure S8: Copy Number Variation (CNV) including *PPFIA3* locus. **(A)** CNV gain and loss involving the *PPFIA3* locus, inheritance pattern, and size. **(B)** CNV losses affecting *PPFIA3* and their phenotypes. **(C)** Reported phenotypes in >2 individuals with *PPFIA3* CNVs in DECIPHER v11.21. **(D)** Reported phenotypes in 2 individuals with *PPFIA3* CNVs in DECPHER v11.21.

SUPPLEMENTAL TABLES

Primer name	Sequence	Purpose
PPFIA3 WT-NS-F	CTATTCCTGCGCGAACCCAGCTTTC	To remove stop codon from the WT cDNA
PPFIA3 WT-NS-R	GTCCGGACCGAAACGCCG	To remove stop codon from the WT cDNA
PPFIA3 p.Arg39Cys-NS-F	GCTCACGGAGTGCGAGC	Mutagenesis
PPFIA3 p.Arg39Cys -NS-R	ATCGTGACCATGAGGCGC	Mutagenesis
PPFIA3 p.Ala315Ser-NS-F	CTACCTGAGCTCCCAGCGGGA	Mutagenesis
PPFIA3 p.Ala315Ser-NS-R	CGCTTCTCCAGTGTTGTAATCC	Mutagenesis
PPFIA3 p.Arg415Trp-NS-F	TCAAGAGCTGCAGTGGGC	Mutagenesis
PPFIA3 p.Arg415Trp-NS-R	TTCTTCTCTTCCAGCTGGGCCTCC	Mutagenesis
PPFIA3 p.Trp546Cys-NS-F	GGGGCCGCTGTTCAGG	Mutagenesis
PPFIA3 p.Trp546Cys-NS-R	TCTTGCCTGCCCGAC	Mutagenesis
PPFIA3 p.Arg784Trp-NS-F	ACCCCCAGGCTGGGACAGCTC	Mutagenesis
PPFIA3 p.Arg784Trp-NS-R	CCCATTCGTCCCTTCTCTTTCTTG	Mutagenesis
PPFIA3 SeqP1	TGTAAAACGACGGCCAGT	Sequencing
PPFIA3 SeqP2	GGAGCGCGTGGCAGTGC	Sequencing
PPFIA3 SeqP3	CGCTCAACAAGGCCGAGGAAC	Sequencing
PPFIA3 SeqP4	GAGCAGCTGGAGGCCATCAAC	Sequencing
PPFIA3 SeqP5	GGGCCTACCTTTTGCTGCC	Sequencing
PPFIA3 SeqP6	CCTGAAGGAATTTGCCACG	Sequencing
PPFIA3 HA-F	TACCCATACGATGTTCCTGACTATG	genomic PCR and qPCR to check HA levels (Figure S2, Figure S4)
PPFIA3 HA-R	AAGTGGCGCGACGCTTAGT	genomic PCR and qPCR to check HA levels (Figure S2, Figure S4)
PPFIA3 cDNA-F	GAACAGCTGTCTAGGCGGCG	genomic PCR and qPCR to check PPFIA3 levels (Figure S2, Figure S4)
PPFIA3 cDNA-R	CCGCAGCTGGCACACCT	genomic PCR and qPCR to check PPFIA3 levels (Figure S2, Figure S4)
rps17-genomic-F	AAGCGCATCTGCGAGGAG	genomic PCR and qPCR endogenous control for PCR (Figure S2, Figure S4)
rps17-genomic-R	CCTCCTCCTGCAACTTGATG	genomic PCR and qPCR endogenous control for PCR (Figure S2, Figure S4)

Abbreviations: wildtype (WT), no stop (NS), forward primer (F), reverse primer (R), sequencing primer (SeqP#)

Table S1: Primer sequences for Q5 mutagenesis, sequencing, and genomic PCR and qPCR

Number of pupae counted for calculating pupal lethality and eclosion defect in Figure 3A							
Set	**empty**	**WT**	**p.Arg39Cys**	**p.Ala315Ser**	**p.Arg415Trp**	**p.Trp546Cys**	**p.Arg784Trp**
1	467	535	444	354	496	127	432
2	238	132	154	173	153	119	193
3	180	143	138	105	159	157	119
Number of adult flies counted for the calculating the abnormal leg morphology in Figure 3B							
Set	**empty**	**WT**	**p.Arg39Cys**	**p.Ala315Ser**	**p.Arg415Trp**	**p.Trp546Cys**	**p.Arg784Trp**
1	231	221	15	109	154	65	190
2	115	62	16	21	71	63	88
3	88	69	7	27	51	82	45
Number of larvae counted for the rescue experiment in Figure 5							
Set	**empty**	**WT**	**p.Arg39Cys**	**p.Ala315Ser**	**p.Arg415Trp**	**p.Trp546Cys**	**p.Arg784Trp**
1	200	1131	457	not tested	691	255	875
2	326	155	192	not tested	239	370	256
3	97	141	170	not tested	114	141	122

Table S2: Sample size for eclosion defects and leg defects in Figure 3, and rescue experiment in Figure 5

Table S3: Statistical summary of data for *PPFIA3* variant studies

Large table, see separate Excel spreadsheet file.

Table S4: General information and clinical findings in individuals 1-10 with *PPFIA3* variants

Large table, see separate Excel spreadsheet file.

Table S5: General information and clinical findings in individuals 11-20 with *PPFIA3* variants

Large table, see separate Excel spreadsheet file.

Table S6: Motor, language, and social milestones in individuals 1-20

Large table, see separate Excel spreadsheet file.

Table S7: Summary of *PPFIA3* variants and transgenic *UAS-PPFIA3* fly alleles

Individual	Human *PPFIA3* variant	Transgenic fly with PPFIA3 variant
1	NM_003660.4:PPFIA3 c.115 C>T (p.Arg39Cys)	UAS-PPFIA3 p.Arg39Cys-HA
2	NM_003660.4:PPFIA3 c.115 C>T (p.Arg39Cys)	UAS-PPFIA3 p.Arg39Cys-HA
3	NM_003660.4:PPFIA3 c.118 G>A (p.(Glu40Lys))	n/a
4	NM_003660.4:PPFIA3 c.239 A>C (p.Gln80Pro)	n/a
5	NM_003660.4:PPFIA3 c.240+1 G>A	n/a
6	NM_003660.4:PPFIA3 c.240+1 G>A	n/a
7	NM_003660.4:PPFIA3 c.943 G>T (p.Ala315Ser)	UAS-PPFIA3 p.Ala315Ser-HA
8	NM_003660.4:PPFIA3 c.1243 C>T (p.Arg415Trp)	UAS-PPFIA3 p.Arg415Trp-HA
9	NM_003660.4:PPFIA3 c.1243 C>T (p.Arg415Trp)	UAS-PPFIA3 p.Arg415Trp-HA
10	NM_003660.4:PPFIA3 c.1285 C>T (p.Arg429Trp)	n/a
11	NM_003660.4:PPFIA3 c.1492 C>T (p.Arg498Trp)	n/a
12	NM_003660.4:PPFIA3 c.1638 G>T (p.Trp546Cys)	UAS-PPFIA3 p.Trp546Cys-HA
13	NM_003660.4:c.2350 C>T (p.Arg784Trp)	UAS-PPFIA3 p.Arg784Trp-HA
14	NM_003660.4:PPFIA3 c.2609 T>A (p.Ile870Asn)	n/a
15	NM_003660.4:PPFIA3 c.2609 T>A (p.Ile870Asn)	n/a
16	NM_003660.4:PPFIA3 c.2706dup (p.Ser903Leu fs*86)	n/a
17	NM_003660.4:PPFIA3 c.2717 C>T (p.Ser906Leu)	n/a
18	NM_003660.4:PPFIA3 c.3307del (p.Glu1103Asnfs*8)	n/a
19	NM_003660.4:PPFIA3 deletion exons 22-30	n/a
20	NM_003660.4:PPFIA3 c.[2377C>A]; c.[2276 A>G] (p.Pro793Thr; pLys759Arg)	n/a

Table S7: Summary of *PPFIA3* variants and transgenic *UAS-PPFIA3* fly alleles

Table S8: PPFIA3 copy number variants (CNVs) in Decipher (11.21), gnomAD SVs (v2.1), and ClinVar (access date July 27, 2023)

Large table, see separate Excel spreadsheet file.

SUPPLEMENTAL ACKNOWLEDGEMENTS

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