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Supplemental Information

Disruption of *POGZ* is Associated with Intellectual Disability and Autism Spectrum Disorders

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Supplemental Case Reports

Individual 1/UMCN1

This female was born at term after an uncomplicated pregnancy and delivery with a normal birth weight of 3,010 grams and normal head circumference of 34 cm. By the age of 12 months her head circumference had declined to -4 SD. She had a severe developmental delay. At the age of 18 months she was able to sit without support. At the age of 5 years, upon her last clinical evaluation, she still needed support while standing and walking. She could speak a few words. Dysmorphic features included hypertelorism, brachycephaly, frontal bossing and midface hyplasia (Figure 1A-B). She was microcephalic. Her behaviour was characterized by stereotypic munching mouth movements, sleeping problems, restlessness and her eye contact was abnormal. Medical concerns included optic nerve hypoplasia, recurrent infections of upper airway and bladder. At the age of 2 years she was operated on an intestinal malrotation. Brain MRI showed a cavum verga cyst, but was otherwise unremarkable. Ultrasound examination of the urinary tract revealed no abnormalities. A metabolic screen revealed normal results. Previous genetic investigations included array-CGH analysis (180k) and DNA-diagnostics of PTPN11, SLC2A1, Angelman syndrome, TCF4, RAI1 and EHMT1. Results were all normal. Whole exome sequencing identified a *de novo* nonsense mutation in *POGZ.* (c.2590C>T; (p.(Arg864*))).

Individual 2/UMCN2

The pregnancy was complicated by recurrent bleeding. At 7 months of pregnancy renal abnormalities were seen on ultrasound examination (left sided hydronephrosis and right sided dysplastic kidney). Birth was uncomplicated and at term. Birth weight was normal (3,00- grams at 37 weeks). He had congenital Horner syndrome and the neonatal period was complicated by feeding difficulties. Developmental milestones were delayed. He was able to sit without support at the age of 2 years and walked without support at the age of 2,5 years. Speech development was more delayed. After the age of 2,5 years he started to make sounds. At the age of his last clinical examination, 9 years, he was only able to speak a few single words. He used pictograms for communication. Medical concerns included recurrent upper airway infections, a severe hypermetropia (+9/+7.5 D) and astigmatism. He was operated on an inguinal hernia and underwent a pyeloplasty. Upon physical examination at the age of 9 years he had a height of 137,5 cm (>25th centile) and a head circumference of cm 49,5 cm (2nd centile). Facial dysmorphic features included brachycephaly, asymmetric facies, flat midface, prominent glabella, prognathism, upslanted palpebral fissure on the right, left sided ptosis, narrow deviated nose with upturned nasal tip and carp shaped mouth with thin upper lip (Figure 1C). Previous (genetic) investigations included a metabolic screen, 250K SNP array analysis and family

based whole exome sequencing. Results were all normal. Subsequent whole genome sequencing in research revealed a *de novo* nonsense mutation in *POGZ* (c.3001C>T; (p.(Arg1001*))).

Individual 3/UMCN3

This male was born as the second child of his non consanguineous parents. Pregnancy and birth were uncomplicated and he was born at term with a normal birth weight of 3,260 grams. The neonatal period was complicated by feeding difficulties (slow to feed), and apathy. Developmental milestones were delayed. Speech delay was more severe than motor delay. At the age of 1 year he was able to sit unsupported, at the age of almost 2 years he was able to walk without support. Since the age of 7 years he is able to speak in simple sentences. A formal intelligence test was done twice. At the age of 3,5 years his IQ as 75-80 and at the age of 7 years 75. His development made good progress and at the age of 9 years he started to read and write simple language. He has had severe feeding problems for which tube feeding was needed from the age of 11 months to 27 months. Up to now the last clinical evaluation at the age of 9 years he only accepted mashed food. His mouth area was very sensitive and he had an aversion to tooth brushing. He had an over-friendly, happy behaviour, needed structure, and was hyperactive. His social skills improved with age. An ASD was suggested (PDD-NOS), but this was not tested formally. Apart from hypermetropia he has no medical problems. At the age of 4 years and 4 months he had a head circumference of 47,5 cm (centile) and a height of 105,6 cm (centile). Upon the last physical examination at the age of 8 years he had a head circumference of 48,8 cm (2nd centile) and a height of 131.7 cm (centile). Facial dysmorphic features were mild included a broad forehead, flat philtrum and thin upper lip (Figure 1D-E). Previous genetic investigations included conventional karyotyping, subtelomere MLPA and 250K SNP array analysis. Results were normal. Whole exome sequencing revealed a *de novo* frameshift mutation in *POGZ* (c.3456_3457del; (p.(Glu1154Thrfs*4))).

Individual 4/UMCN4

The pregnancy was complicated by polyhydramnion. Birth was uncomplicated and at term. He had a normal birth weight of 3,950 grams. The neonatal period was complicated by excessive crying and feeding difficulties. Later on he showed few initiative and was very quiet. His motor development was delayed and he had hypotonia. At the age of 2 years he was able to walk without support. His motor skills improved, but he was hindered by hypermobility of his joints. Speech development was delayed. He started talking since the age of 4,5 years. Since then he made good progression. He had behaviour problems suggesting an autism spectrum disorder, including tics, sticking to routines, and obsessions. This was not formally tested. In addition he was over-friendly and mainly focused on contact with adults. He has sleeping problems, for which he was on Melatonin medication. At the last

clinical evaluation when he was 5 years old, he had still some problems with feeding, including chewing difficulties and aversion to cold and hot food. His mouth area was very sensitive. His hearing and vision were normal. As an infant he had recurrent infections, including upper airway and bladder. At the age of 5,5 years he had twice a severe metabolic acidosis during gastro-enteritis. Brain MRI at the age of 2 years was normal. Upon physical examination at the age of 5 years and 2 months he had a head circumference of 49 cm (<16th centile) and a height of 108,5 cm (16th centile). He had no facial dysmorphic features, except for a somewhat small mouth with thin upper lip (Figure 1F-G). Previous (genetic) investigations included a metabolic screen, 250K SNP array analysis, DNA-diagnostics of Angelman syndrome, the *DMPK* gene and the *MED12* gene. These revealed no explanation. Whole exome sequencing identified a *de novo* frameshift mutation in *POGZ* (c.2263del; (p.(Glu755Serfs*36))).

Individual 5/UMCN5

The pregnancy was uncomplicated. Delivery was by secondary Caesarean section. He had a good start and high birth weight of 4,000 grams at 38 weeks of pregnancy. Neonatal period was complicated by a perinatal infection. His development was delayed from the beginning. He had hypotonia and was able to walk with support at the age of 2 years and 4 months. He started babbling at the age of 2 years and 4 months. At the age of 2 years and 4 months he had a developmental age of 10-12 months. His behaviour was happy and friendly and he had tics. Sleeping was problematic. At the age of 3,5 years he learned to use sign language and was able to speak a few single words. He could walk without support, but his gait was unsteady and clumsy. During his first years of life he had recurrent airway infections. At the age of 1 year he had a severe airway infection with respiratory insufficiency and was admitted to the intensive care unit. He had sleeping problems and obstructive sleep apnea syndrome. Feeding was complicated by swallowing problems and he was not able to eat solid food. His mouth area was very sensitive. At the age of 11 months he had a microcephaly (head circumference 41 cm). He had a normal height and weight. Weight increased over time to the 99th centile at the age of 2 years. Hearing was normal. He was diagnosed with hypermetropia and alternating exotropia. During a period of fever he had a single seizure. Brain MRI performed at the age of 5 months showed slightly prominent peripheral and central liquor spaces in the frontal regions and slightly delayed myelinsation of the white matter. Facial dysmorphic features included hypertelorism and brachycephaly . Previous (genetic) investigations included a metabolic screen, array analysis and DNA-diagnostics of *TCF4, CHRNE* and *RAPSN.* Array analysis revealed a maternally inherited 224 kb deletion in chromosomal region 16p11.2 (28,8 - 29,0 Mb; Hg 19). Mother was healthy. Because of his severe phenotype that could not be explained by the 16p11.2 deletion whole

exome sequencing was performed. This revealed a *de novo* frameshift mutation in *POGZ* (c.1152dup; (p.(Arg385Serfs*4))).

Individual 6/UMCN6

This male was born at term after an uncomplicated pregnancy and delivery. His birth weight was normal (3,280 grams). He was able to walk without support at the age of 14 months. Fine motor skills were weak. Speech development was delayed. He started to speak his first words after the age of 3 years. He was not able to follow regular education and moved to a special school. A formal IQ test showed an IQ in the range of mild ID (IQ 66). At the age of 11 years he started to read and write simple language. His behaviour was over-friendly and he was diagnosed with an autism spectrum disorder (PDD-NOS) and Attention Deficit Hyperactivity Disorder. He was operated on undescended testes and underwent a tonsillectomy and got ear tubes. He needed glasses because of hypermetropia (+4, 5 D). He had a tendency to overeat and to become overweight. Upon physical examination at the age of 11 years he had a height of cm 156,5 cm (70th centile), weight of 59, 4 kg (> 98th centile) and head circumference of cm 54,5 cm (60th centile). Facial dysmorphic features included a high forehead, hypertelorism, almond shaped palpebral fissures, ptosis and overfolded helices of the ears (Figure1H-I). He had tapering fingers with hyperlaxity of the joints. 250K SNP array analysis showed normal results. Whole exome sequencing revealed a *de novo* mutation in the donor splice site of intron 16 of the *POGZ* gene (c.2432+1G>A; (p.?)).

Individual 7/UMCN7

This female was the first child of healthy, non-consanguineous parents. She was born at 38 weeks. Delivery was difficult because of slow progress, but she had a good start after birth. She was a very quiet baby, not asking for attention. Because of plagiocephaly she was treated with a helmet. Her general development was slow. At the age of 23 months her mental and motor development were estimated to be at 15 months. She lost words and had stereotypic movements with her hands. There was a lack of eye contact and an evident language development delay. At almost 5 years of age developmental age was conform 3 years and 11 months, which was higher than in a former test. She was diagnosed with an autistic spectrum disorder. Upon physical examination at the age of 4 years she had a height growth at the 50th centile. Her head circumference was at the 2nd centile and possible declining compared to former measurements. She was a pleasant and quiet girl. She was looking up and made little eye contact. There were no apparent facial dysmorphisms (Figure 1J). Previous genetic investigations included array analysis and DNA-diagnostics of *FMR1, MECP2* and *TCF4.* Result*s* were all normal. Whole exome sequencing identified a *de novo* frameshift mutation in *POGZ* (c.2020del; (p.(Arg674Valfs*9))).

Individual 8/UMCN8

This male was born at term after an uncomplicated pregnancy and birth. His parents are from Azerbaijan. He had a low birth weight of 1,400 grams. In the neonatal period he cried excessively. He was able to walk at the age of 1,5 years. He started to talk after the age of 2 years and was able to speak short simple sentences. During puberty he lost language skills and was no longer able to speak in sentences and used a few single words. His behaviour is characterized by anxiety, self mutilation, sometimes aggression and sleeping problems. He had problems with chewing and did not like to eat solid food. An EEG at the age of 20 years, performed because of his behaviour problems, showed abnormalities in the frontal regions, suggestive for epileptic phenomenon's. However further EEG examinations were not conclusive for epilepsy. At the age of 26 years he had a height of 167 cm (0,6th centile), weight of 85 kg (>98th centile) and head circumference of 54 cm (1st centile). Facial dysmorphic features included brachycephaly, a high nasal bridge and slight deviation of the nose, upturned tip of the nose and thins upper lip (Figure 1K-L). Previous (genetic) examinations included array analysis (CytoScan HD analysis) and a metabolic screen. Results were normal. Whole exome analysis revealed a *de novo* nonsense mutation in *POGZ* (c.3847C>T; (p.(Gln1283*))).

Individual 9/UMCN9

This male was born at term after an uncomplicated pregnancy and delivery. His father is originally from Korea. He was a quiet baby. Psychomotor development was delayed. He was able to walk without support at the age of 2 years. Speech and language development was more delayed, but at the age of 8 years he was able to talk in sentences and could write and read a few single words. At the age of 6 years a formal intelligence test was performed and showed an IQ of 55. His behaviour was hyperactive and clownish. During the first 1,5 years of his life he had recurrent upper airway infections. He had hearing problems due to recurrent middle ear infections with effusion and got ear tubes. He was diagnosed with a high hypermetropia (+5 D). EEG investigation showed abnormalities suggestive for epileptic phenomenon's, but he never had clinical seizures. On treatment with Keppra his cognitive performance improved. One of the brothers of mother and a son of another brother of mother have epilepsy and a normal intellectual development. Upon physical examination at the age of 8 years he had a height of 129 cm (30th centile), weight of 35,4 kg (>99th centile) and head circumference of 52 cm (40th centile). Facial dysmorphic features were mild and included brachycephaly, flat midface, hypertelorism and epicanthic folds (Figure 1M). Previous (genetic) investigations included a metabolic screen and 250K SNP array analysis. Results were normal. Whole exome sequencing identified a *de novo* frameshift mutation in *POGZ* (c.3456_3457del (p.(Glu1154Thrfs*4)). In addition he had a maternally inherited missense mutation in the gene

ATP1A2 (c.1975G>T; (p.Ala659Ser)). Mutations in *ATP1A2* have been reported in individuals with hemiplegic migraine (OMIM #602481) with or without epilepsy and sporadically in individuals with only epilepsy[1]. It may be that the mutation in *ATP1A2* contributed to the phenotype of the individual with respect to the epileptic phenomenon on his EEG, but this is uncertain.

Individual 10/UMCN10

This female was born at term after an uncomplicated birth. Pregnancy was complicated by maternal diabetes mellitus and hypothyroidism. Birth weight was with 6,070 grams very high. The neonatal period was complicated by hypoglykemia and feeding difficulaties, for which she got tube feeding. Initially growth normalized. After the age of 9 years she had an increase in weight and got obese (Figure 1N-O). At the age of 3 years speech and motor delay were evident. At the age of 10 years a formal IQ test was performed. This resulted in an IQ of 55. She was social, hyperactive and had attention problems. As a young child she had frequent middle ear infections. A *de novo* nonsense mutation in *POGZ* was identified in this individual (c.3040C>T; (p.(Gln1014*))).

Individual 11/EE1

This individual is an 8-year-old Caucasian male. Individual was diagnosed with autism spectrum disorder (confirmed with ADOS, ADI, and clinical judgment using DSM-IV criteria). He primarily speaks in complex sentences. Individual shows autism-related impairments in social communication, including very little social reciprocity, limited social response, impaired conversation skills, poor eye contact, and limited insight into emotions and social relationships. Individual has a strong history of restricted interests, repetitive play, compulsive behaviors, difficulty with change, sensory interests, and self-injurious behavior. Individual's cognitive and adaptive abilities fall in the mildly impaired range (Verbal IQ = 88, Nonverbal IQ = 73, Full Scale IQ = 75, and Adaptive Composite = 65). Individual had a significant speech delay, with first single words at 48 months of age and first phrases at 58 months. Individual was also delayed in walking and took his first independent steps at 28 months. Abnormalities were first noted in his development at 12 months of age. Individual's parents endorse significant internalizing (anxiety, depression) and externalizing (attention problems, aggression, defiance) behavior problems. Individual has average receptive vocabulary (PPVT Standard Score = 96) and below average fine motor coordination (Purdue Pegboard T scores, Dominant = 37, Non-dominant = 31, Both Hands = 39). Individual has average head circumference (52.9 cm, z = -0.20). He is of average height (131.3 cm, z = -0.03) and above average weight (35 kg, z = 1.28), with a BMI indicative of obesity. Individual was born vaginally at 40 weeks gestation and had a nuchal cord. Mother reported that individual was overly lethargic as an infant. Individual is ambidextrous and has unspecific vision problems that are corrected. Individual has no reported gastrointestinal

disturbances or neurological problems. He has a significant history of otitis media (>8 infections) and strep throat diagnoses. Individual also has a history of difficulty breathing at night, and his tonsils and adenoids were removed at 7 years of age. He experiences frequent night-time awakenings and sleepwalks at night, and his parents report that he is excessively tired during the day. Individual experiences night-time enuresis. Whole exome sequencing identified a *de novo* frameshift mutation in *POGZ* (c.3600_3607dupTGATGACG; (p.(Glu1203Valfs*28))).

Individual 12/EE2

This individual is a 14-year-old Caucasian male. Facial features include prominent midface with tubular shaped nose, bilateral epicanthus, and slightly posteriorly rotated ears (Figure 1T). Left first digit of the hand has lateral deviation of distal phalanx and slightly tapered digits are found in all 10 fingers. Excessive laxity in metacarpophalangeal joints is also noted. Physical examination reveals obesity and slight gynecomastia. Individual has a historical diagnosis of microcephaly and, per parental report, had an MRI at 3 years of age indicating a "very small brain." Individual currently has an above average head circumference measurement (*57 months:* HC = 49.9 cm, *z* = -1.29; *9 years:* HC = 52.5 cm, *z* = -0.71; 14 years: HC = 56.6 cm, *z* = 1.01). Head circumference measurements are unavailable prior to 57 months. Individual has history of average height and above average weight measurements, with BMIs indicative of obesity in recent years (*Birth*: height = 50.8 cm, *z* = 0.31, weight = 3.4 kg, *z* = -0.24; *57 months*: height = 104.5 cm, *z* = -0.67, weight = 19.4 kg, *z* = 0.60; *9 years*: height = 133 cm, *z* = -0.65, weight = 51.6 kg, *z* = 2.16; *14 years*: height = 163 cm, *z* = -0.36, weight = 90.3 kg, *z* = 2.43). Individual was diagnosed with autism spectrum disorder (confirmed with ADOS, ADI, and clinical judgment using DSM-IV criteria) and mild intellectual disability (confirmed with cognitive and adaptive testing). He primarily speaks in complex sentences, but continues to make grammatical and articulation errors, and has a concurrent diagnosis of Speech Sound Disorder. Individual shows autism-related impairments in social communication, including unusual intonation to speech, very little social reciprocity and social commentary, limited conversation skills, atypical eye contact, and limited insight into social relationships. No repetitive/scripted speech, sensory interests, repetitive behaviors, or complex motor mannerisms were observed during his evaluation at 14 years of age. However, individual has a strong history of unusual preoccupations, restricted interests, difficulty with change, sensitivity to loud sounds, and complex motor mannerisms. Individual's cognitive and adaptive abilities fall in the impaired range (Verbal IQ = 84, Nonverbal IQ = 63, Full Scale IQ = 70, and Adaptive Composite = 72). Individual first used single words at 9 months of age, and first phrases at 24 months. Abnormalities were first noted in his development at 6 months of age. Executive functioning skills are impaired (DKEFS: Verbal Fluency, Design Fluency, and Color-Word Interference Scaled Scores all <6). Individual has low average receptive vocabulary (PPVT

Standard Score = 89) and average expressive vocabulary (EVT Standard Score = 96). He also has significantly impaired fine motor coordination (Purdue Pegboard T scores all <10 and Movement ABC Manual Dexterity Scaled Scores = 1) as well as difficulty with gross motor coordination (Movement ABC Aiming & Catching subtest Scaled Score = 1 and Balance subtest Scaled Score = 4). Parent report about his social responsiveness on the SRS-2 suggests severely impaired restricted interests and repetitive behavior, social awareness, social cognition, moderate impairment in social communication, and no impairment in social motivation. Individual was born vaginally at 40 weeks gestation following a labor that was augmented by Pitocin due to prolonged ruptured membranes. Individual had a nuchal cord and was jaundiced at birth, but no treatment was given. Physical anomalies were noted in his hands at birth. Individual has a history of low muscle tone that, according to parent report, resolved at 8 years of age. He experiences constipation, but has no other gastrointestinal disturbances. He has been diagnosed with sleep apnea and wears a CPAP machine at night. No other sleep disturbances are noted. Individual has enuresis and encopresis, but no other psychiatric problems are noted. His temperament, cognition, social interaction, and communication skills appear to improve when he has a fever. Whole exome sequencing identified a *de novo* nonsense mutation in *POGZ* (c.3022C>T; (p.(Arg1008*)))[2].

Individual 13/EE3

This individual is a male, currently aged 27, but seen last time at age 19 years. Nothing relevant in his family history, nor in his pregnancy. Eutocic delvery at 40 weeks, low birthweight (2,700gr) with mild cyanotic neonatal asphyxia. Too much quiet and rarely crying during first months of postnatal life, he was delayed in his psychomotor milestones since the very beginning. Since infancy he showed impairments in social relations, and since 10 years appeared irritable and sometimes heteroaggressive. Brain MRI, performed at age 18 years, showed small gliotic spots over and under the tentorium. Karyotype and 44k arrayCGH have been normal. Clinical phenotype shows obesity, asymmetric face, small ears with wide concha, and short toes. At the neurologic examination deep tendon reflexes were decreased in the upper limbs and increased in lower limbs, the gait was awkward. Ophthalmologic evaluation showed astigmatism in both eyes, with hyperopia in the right one and amblyopia in the left one. Intolerance to carbohydrates was unveiled by OGTT. Normal results came from heart auscultation and EKG, audiometry, EEG, routine blood tests. Psychometric testing with WAIS-R and Raven's SPM highlighted a mild intellectual disability with higher performance than verbal IQ. No behavioral psychopathology was appreciated during the evaluation. Targeted sequencing by molecular inversion probes identified a *de novo* frameshift mutation in *POGZ* (c.2196_2198delAG; (p.(Val733del l))).

Individual 14/EE4

This individual is a currently 19-year-old male, seen by us for the first time at age 12 years. Nothing in the family history, not consanguineous parents, IUGR, delivery by CS after 37w pregnancy. BW 2250gr. No asphyxia nor jaundice. Delayed psychomotor milestones since the very beginning. Poor school performance at 6 years prompted psychometric evaluation, with resulting assessment of ID. Speech and psychomotor treatment was immediately started. Allergic conjunctivitis appeared in the summer season. At our first evaluation at age 12, he was in good health, with normal head circumference and growth parameters, without apparent dysmorphic features and neuromotor impairments (Figure 1P). We confirmed allergic conjunctivitis, and its allergene. Hypermetropia was present in both eyes. Normal results: routine blood tests, brain CT scan, audiometry. Psychometric evaluation with WISC-III, Leiter-R and VABS assessed a mild degree of Intellectual Disability. His behavior during testing was quiet, passive, with poor curiosity and interest. He showed performance anxiety and scarce self-esteem, but pursued all the items. He showed impairments in planning and self-organization. Socio-affectivity was immature and dependent on the mother, but coherent with the degree of ID. He showed an adequate knowledge of social rules and, after a starting shyness, showed a correct relational ability. The mood was peaceful. Performance IQ was lower than verbal IQ. If stimulated, he can set up a communicative approach, and brief reports with poor content. First follow-up check at age 13 confirmed the higher adaptivity vs. the lower cognitive performances, which places the individual at the mild/moderate ID border. He showed mild shyness, but was relationally adequate. Mood was stable and eye contact was correct. Attention times were enough for ending the testing. Speech was adequate to the context, but content was immature. The independence improved, becoming less dependent on the mother and the home. Basic self-autonomy is attained, he's tidy and routinely performer. WISC-III retesting assessed an increased Verbal IQ>Performance IQ unbalance. Follow-up check at age $14^{7/12}$ highlighted a behavioral shift toward obsessive compulsive disorder (OCD). He reacted to change with rage, and only when he could go ahead with his routines he was calm, quiet but slow in achieving goals. Array CGH was normal. Last evaluation at age 16 confirmed mild ID and obsessive compulsive traits. Indeed, he improved in his capability to shift from his routines, decreasing the numbers of rage behaviors. Targeted sequencing by molecular inversion probes identified a *de novo* frameshift mutation in *POGZ* (c.2020del; (p.(Arg674Valfs*9))).

Individual 15/EE5

This individual is female and was 14 years of age at the time of the behavioral evaluation. At a later assessment at 21 years of age, the individual was of low average height (height = 157 cm, z = -0.98)

and above average weight (weight = 73.9 kg, *z* = 1.2) with low average head circumference (HC = 53.5 cm, z = -0.79). Individual was diagnosed with Autism Spectrum Disorder and has adaptive skills in the very impaired range (Adaptive Composite = 41). She currently speaks in 2-3 word sentences, uses frequent echolalia, and has impaired nonverbal communication (limited eye contact, facial expression, and gestures). She has some repetitive motor mannerisms and strong circumscribed interests that impede on daily activities. Individual first used words at 36 months of age and first phrases at 48 months. Abnormalities were first noted in her development at 12 months of age. Targeted sequencing by molecular inversion probes identified a *de novo* nonsense mutation in *POGZ* (c.1212C>A; (p.(Tyr404*))).

Individual 16/EE6

This individual was born in due course of gestation to healthy, non-consanguineous Chinese parents as first child after a normal pregnancy (Figure 1U). Her birth weight was 5.5 Kg, height 51 cm. On examination at 7 years of age, her height was 117 cm (z=-1.25), weight 19 kg (z=-1.67), and head circumference 48.5 cm. (P5-P10). She was diagnosed with Autism Spectrum Disorder (met criteria on ADI-R and the DSM-IV-R) and mild Intellectual Disability. She had limited social interactions, and communication difficulties. She did not have any regression. She had repetitive motor mannerisms (e.g., spinning her body) and stereotypic interests (e.g., toilet bowl and water cup). She spoke no meaningful words and could understand simple commands but not complex sentences. She demonstrated obsessive behavior. For example, she always required laying out items at the table, always required wearing boots. Her score on the Aberrant Behavior Checklist was 81 and her total score on the Social Responsiveness Scale was 118. She had seizures on two occasions: the first time at 2 years of age, the second time at 3 years of age. Parents reported that her EEG is abnormal tested after the seizure. Parents reported that the brain magnetic resonance imaging showed no structural anomalies. G-banded karyotyping was normal (46, XX). Targeted sequencing by molecular inversion probes identified a *de novo* nonsense mutation in *POGZ* (c.538C>T; (p.(Gln180*))).

Individual 17/EE7

This boy is the first child, born to healthy non consanguineous parents originating from Turkey (Figure 1V). He has three younger brothers. In addition, the mother has a daughter from a previous relationship. All siblings are healthy and there are no cases of intellectual disability or autism in the family. The child was born at full term after an uneventful pregnancy and delivery. The mother reports though that she experienced severe nausea and malaise during the pregnancy - symptoms that were not present in pregnancies with the siblings. Birth parameters were within the normal range. A delay in motor development was noted during the first year of life - he walked

independently at the age of 20 months. He is now described as clumsy. Cognitive development is delayed. He has speech problems, difficulties with pronunciation and also understanding. Evaluation of his intellectual abilities at the age of 7 years resulted in a diagnosis of mild ID, with a total IQ score of 66. His results at different parts of the cognitive testing were quite even. He attends a regular class but he has a personal tutor. The mother reports that, during the first months of life, the boy was sleeping a lot and was easy to handle. From around 6 months of age the boy is described as extremely happy in combination with being impulsive and having temper tantrums. Furthermore, he is hyperactive and interaction with others is unreserved – even with strangers. He likes to hug people. He does not always realize dangers and often puts himself at risk for hurting himself. He is also prone to run away from his guardians. In addition to all these more or less problematic behaviors he is also described as a content and empathic person. He has sleeping problems. He falls to sleep very late (around midnight) and wakes up frequently during night. At several occasions he has tried to leave the house and run away at night. Neuropsychiatric evaluations at the age of 5 and 7 years, respectively, have resulted in the following diagnoses according to DSM-V: Autistic syndrome, ADHD and Oppositional Defiant Disorder. The growth chart shows normal length and weight at +3SD. The boy wants to eat all the time and does not seem to get full. A moderate bilateral hearing deficit was noted early and the boy now has hearing aid. He has had tube insertion, due to otitis media with effusion. He has strabismus and wears glasses. Two café-au-lait spots were reported, measuring 3x4 cm and 1x1 cm respectively. He has mild pes planus and pes valgus. Targeted sequencing by molecular inversion probes identified a *de novo* nonsense mutation in *POGZ* (c.3139G>T; (p.(Glu1047*))).

Individual 18/EE8

This girl is the second child born to healthy non consanguineous parents originating from Sweden. She has an older sister. From previous relationships, the mother and the father have two and three children, respectively. All siblings are healthy and there are no cases of intellectual disability or autism in the family. The child was born at full term after an uneventful pregnancy and delivery. The mother reported nausea during the first and second trimester. Birth parameters were within the normal range. Motor development was delayed, she walked independently at 18 months, and the girl is now described as being clumsy. Speech development was also delayed, but at present age her verbal skills are near normal. Cognitive evaluation at the age of six years revealed an IQ level of around 80, but with an uneven profile. Best results were seen with tasks requiring verbal skills, which contrasted to low results during tests requiring executive speed. The girl attends a regular class at school. At the moment she does not have extra tutoring, but the parents report that she would actually need more assistance in school. The girl is described as having a happy personality with

mood instability, impulsivity and temper tantrums. She is stubborn and has experienced problems with conflicts during interaction with peers as well as with adults. She is outgoing and unreserved with people, but in an inappropriate manor resulting in difficulties with social interaction. The behavioral problems have improved over time though. She is also described as an empathic, sensitive and loving person. She is physically very active and lacks the ability to concentrate on a task/activity for more than short periods of time. She sleeps well, but requires company during night. If let alone she wakes up frequently. A neuropsychiatric evaluation was performed at the age of four years and six years respectively. At the primary evaluation she did not fulfill the criteria for any diagnosis according to DSM-V. A repeated evaluation two years later resulted in a diagnosis of atypical autism. She did not fulfill the criteria for ADHD or any other diagnosis. The consumption of food is not excessive when compared to the rest of the family. All other members of the family are of normal weight. Persistent otitis media with effusion occurred requiring bilateral tube insertion at the age of five years with normal hearing. She has hyperopia and astigmatism requiring glasses. Targeted sequencing by molecular inversion probes identified a *de novo* frameshift mutation in *POGZ* (c.2291del; (p.(Pro764Leufs*27))).

Individual 19/FR2

This female was the first child of her parents, she has three healthy brothers. There was no relevant family history. She was born at term after an uncomplicated pregnancy, by caesarean section at 37 WG. Birth weight was 2900 g (-1 SD), birth length 48 cm (-1.5 SD) and OFC 33 cm (-1.8 SD). She presented with normal psychomotor development, walked at 17 months, but had speech delay. She had learning difficulties and went to special school at the age of 7 years old. She had no behavioural issues. She was referred to the Genetics Department at 10 years of age for evaluation of a syndromic overweight. Weight was 35 kg (+1.2 SD), height 138.5 cm (+0.8 SD) and BMI 18.4 (+1.8 SD). At last examination at 11 years and 5 months, weight was 35 kg (+1.2 SD), height 138.5 cm (+0.8 SD) and OFC 52 cm (-1 SD). She had no facial dysmorphism, no neurological abnormality. Targeted sequencing by SureSelect capture identified a *de novo* frameshift mutation in *POGZ* (c.2400dup; (p.Lys801Glnfs*7))).

Individual 20/FR1

This male was the fourth child of non-related healthy parents. His three brothers were healthy. The third trimester of the pregnancy was complicated by intrauterine growth retardation. He was born at term. Birth weight was 2750 g (-2 SD), height was 49 cm (-1 SD), OFC was 32 cm (-2 SD). He was admitted to the neonatology ward for hypotonia and feeding difficulties with failure to thrive. Initial investigations revealed a congenital heart defect (ASD) and a common mesentery. His evolution was

marked by severe psychomotor and speech delay. At last examination at the age of 3 years and ten months, he could crawl but not walk unaided. He was microcephalic (OFC -4 SD), with a normal weight and height. He had no meaningful word, very little social interaction and made no eye contact. He had stereotypic movements, self aggressive behaviour. He wore hearing aids for sensorineural hearing loss. Facial features were marked by a round and flat face, short palpebral fissures with epicanthus, and a tented upper lip with downturned corners of the mouth. He had a micropenis and a bilateral cryptorchidism. Eye examination showed a cherry red spot and he developed aspecific keratitis. All the metabolic investigations were normal. Cerebral MRI revealed a delayed myelination. Whole exome sequencing identified a *de novo* frameshift mutation in *POGZ* (c.2545+1del; (p.?)).

Individual 21/FR3

This female is the child of second-cousin parents (Figure 1Q-R). Family history was otherwise unremarkable. The pregnancy was marked by polyhydramnios and the diagnosis of bilateral ureteral bifididy and cyst of the septum pellucidum. Karyotype was normal. Birth weight was 3290 g (+0.5 SD), length was 49 cm (-0.5 SD), OFC was 33 cm (-2SD) at 40 WG. She had neonatal hypotonia and feeding difficulties with gastroesophageal reflux. At the age of 6 months was noted a nystagmus and a strabismus. She was referred to the geneticist at 7 months because of psychomotor retardation. She walked at 29 months and speech was severely delayed. She had sleep disturbance. She attended normal school with support at 6 years. She had severe constipation. On last examination at 6 years, height and weight were on the medium range, OFC was -3 SD. She had a bifid uvula, a tented upper lip, a flat face with prognathism and short palpebral fissures with bilateral epicanthus. Whole exome sequencing identified a *de novo* frameshift mutation in *POGZ* (c.2836del; (p.(Asp946Metfs*12))).

Individual 22/FR4

This individual is a girl with intellectual disability and is the first child of non-consanguineous Caucasian parents (Figure 1S). She has an unaffected younger sister and no noticeable familial medical history is reported. The pregnancy was complicated by mild gestational diabetes. The mother reported poor fetal movements during pregnancy. The child was born by assisted vaginal delivery with forceps at 34.5 weeks. At birth, height (45 cm, 50^{th} percentile) and weight (2310 kg, 50^{th} percentile) were in normal range while a microcephaly was reported (OFC = 29.5 cm, 3^{th} percentile). APGAR scores were 6 at 1 minute and 9 at 5 minutes. She presented a moderate respiratory distress in the first hours of life requiring a transfer to a neonatal intensive care unit. She presented a transient and moderate oxygen dependency, while many episodes of apnea persist for 6 days. During the neonatal period, she had a low reactivity, low spontaneous mobility, global

hypotonia, absence of visual tracking and eating difficulties requiring nasogastric tube feeding. Global hypotonia with an unbalanced tone persisted during the next months, and motor development was delayed: she was able to sit without support at the age of 13 months and she walked independently at 30 months, but with a gait ataxia. She was unable to speak and used pictograms to communicate. Her behavior were characterized by moderate self-mutilation and sometimes aggressive behavior (in distress situation), poor eye contact, relatively little social reciprocity, restricted interests and rare stereotypies. An autism spectrum disorder (ASD) was evoked but this was not tested formally. She did not present sleep disorders. Brain MRI performed at the age of 5 months (and 6 years) showed a cortico-subcortical atrophy and a periventricular leukomalacia. These data are consistent with the neurologic aftermaths of preterm birth. At the later assessment, at the age 9.5 years, she still has no speech. Upon physical examination, her height is 131 cm (- 0.3 SDS), weight 31 kg (+ 1 SDS), and head circumference 49 cm (- 2 SDS). Craniofacial dysmorphic features include an occipital plagiocephaly, malar hypoplasia, tented upper lip, short nose, upturned nostrils, and depressed nasal bridge. Medical problems include constipation, mild myopia (+ 2 d) requiring glasses and a persistent drooling. Despite the main hypothesis of an acquired perinatal cause (premature and hypoxia), the severity of neurological symptoms (severe intellectual disability and possible ASD) and dysmorphic features justified further etiological explorations. A metabolic screening revealed normal results. Previous genetic investigations included conventional karyotype, array-CGH analysis (44k) and DNA-diagnostics of Angelman syndrome and MECP2. Results were all normal. Targeted high-throughput sequencing of 275 genes identified a *de novo* frameshift mutation in *POGZ* (c.2574del; (p.(His858Glnfs*13))).

Individual 23/FR5

This individual the 4th child of young, unrelated and healthy parents. He has 3 healthy sisters and one young brother with intellectual deficiency and autism. We met him for the first time at the age of twenty when he came from Cameroun to France. He was born at term after a normal pregnancy. He grew up, showing a psychomotor retardation. He walked at the age of two and spoke with delay. At the age of twenty he can say some short and very simple sentences. He can't read or write. He presents a severe intellectual disability and he's only able to perform some simple actions of the everyday life (e.g., eating alone, dressing, washing himself). He never presented behavioral trouble, especially no autism traits. He's shy but he likes contact with others. He started a generalized epilepsia at the age of four. He was treated with Valproate leading to a lasting interruption of crisis. At the examination at the age of twenty, He presented a tall stature, clearly above his target stature. He also presented a macrocephaly (+ 3.5 SD) and some dysmorphic features such as a large mouth and an middle face retraction. At neurological examination, we noted an akinesia without

extrapyramidal rigidity. All morphological explorations were normal. The cerebral MRI showed a non specific global atrophia. The ophthalmologic investigations (ocular fundus, electroretinogram, visual evoked potentials) do not find any abnormality. Targeted sequencing using a Illumina TruSight One panel identified a *de novo* nonsense mutation in *POGZ* (c.1810G>T; (p.(Glu604*))).

Individual 24/FR6

This individual is a male born at full term after uneventful pregnancy and delivery. At the time of conception, his healthy mother and father were 37 and 53 years old, respectively. He had an Apgar score of 9, a birth weight of 4 kg and a normal head circumference of 35 cm. He had a global developmental delay with autistic features. He was able to walk at 2 years and could say few words only at 4. At 3 years old, he started to show some ataxic features associated with oculomotor apraxia, without pyramidal syndrome or epilepsy. He had blond hair with a red streak at the fronto-occipital level, as well as a hyper-pigmented skin patch on the shoulder. His behavior was characterized by stereotypic movements, difficulties getting to sleep and crisis with inappropriate laughing and agitation. Brain MRIs demonstrated a thick corpus callosum without other anomaly at the sustentorial level. A cerebellar dysplasia was identified, involving the inferior part of both hemispheres. Spectroscopic analysis detected a small lactate peak in the left lentiform nucleus. Previous investigations with negative results included screening for Fragile X Syndrome, *SHANK3* deletion, CDG syndrome, and genomic disorder by CGH array analysis (Agilent 60k). Whole exome sequencing (WES) was performed using HiSeq2500 (Illumina) and SureSelect All Exon 50 Mb capture kit (Agilent) with 98% coverage of at least 15X. WES detected a heterozygous de novo, variant in the *POGZ* gene located at position c.3001C>T (NM_015100.3) and predicted to truncate the protein at position p.Arg1001*. This de novo variant was validated by Sanger sequencing using the affected case and parents' DNAs.

Individual 25/EE9

This male individual was referred for genetic, karyotype and Fragile X, investigations at the age of 11 months when he was assessed by a pediatric specialist as having delay in his development, facial dysmorphism and bronchiolitis. Karyotype and Fragile X investigations were negative. His mother suffered from a schizoaffective personality disorder and was unable to look after him. This individual was subsequently lost to follow-up. Targeted sequencing by molecular inversion probes identified a frameshift mutation in *POGZ* (c.2501del; (p.(Leu834Trpfs*20))). Inheritance of this event is unknown as parents were not available for testing.

Supplemental Information

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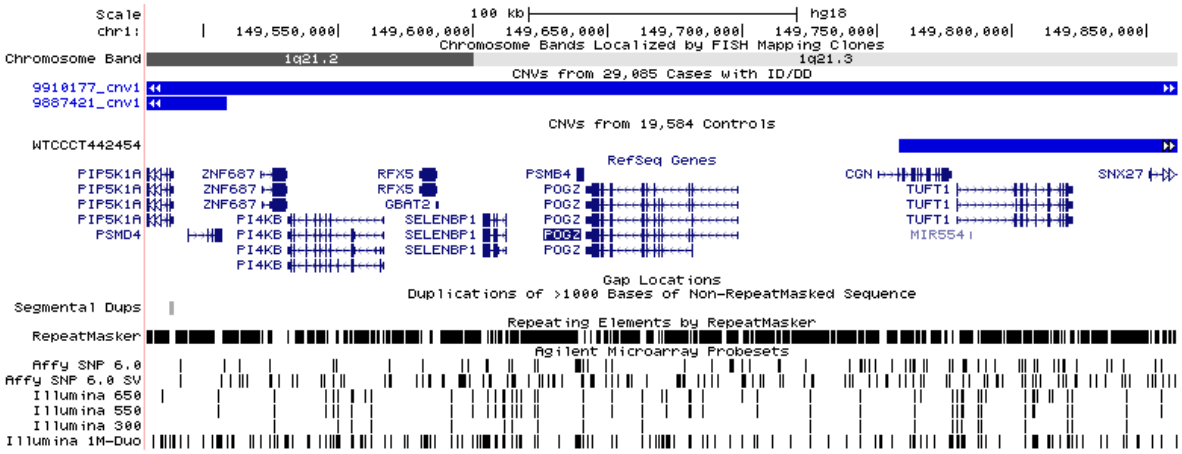
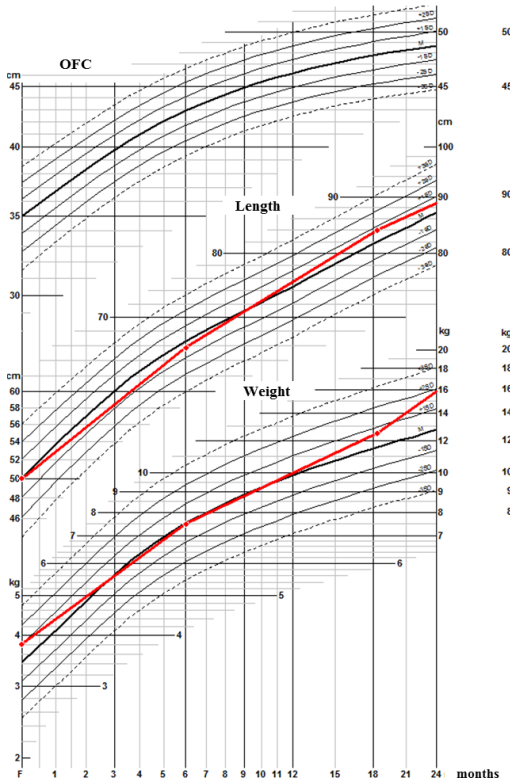
During revision of this manuscript another study reporting five unrelated ID individuals was published by White and colleagues[3]. Their descriptions of the phenotypic features are largely in agreement with our data.

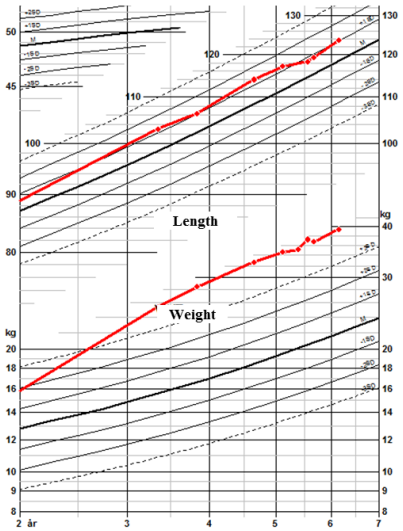
Supplemental Figures



Supplemental Figure S1. CNVs intersecting *POGZ*. Examination of *POGZ* in the context of a CNV morbidity map generated from 29,085 individuals with ID/DD and 19,584 population controls identifies only a single overlapping 8.3 Mbp duplication (blue bar) in an affected individual and no events in controls[4].

A





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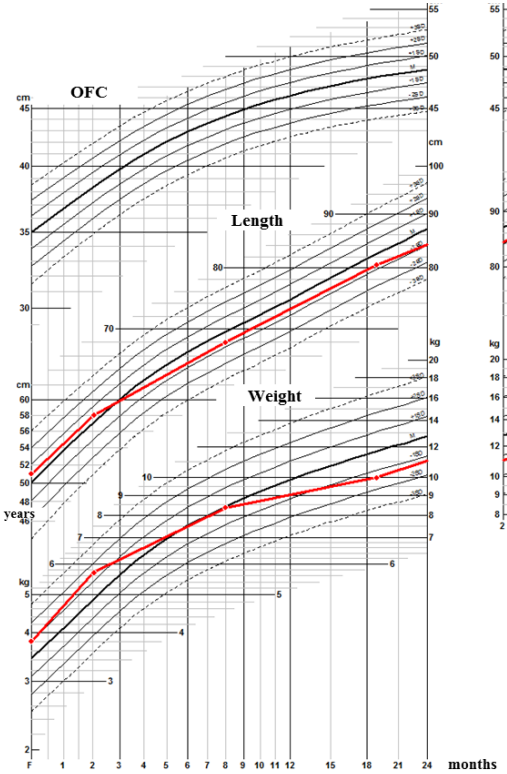

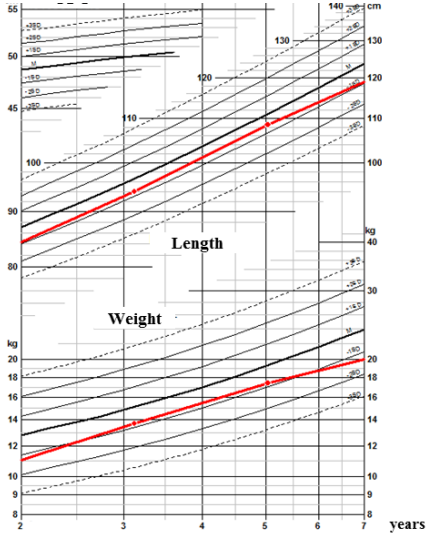

Supplemental Figure S2. Longitudinal physical measurements of one proband-sibling pair show obesity is not likely due to environment. Physical measurements collected from **(A)** individual EE8 and **(B)** her unaffected sibling from birth to seven years of age shown in red. OFC: head circumference (not reported). Length is measured in cm and weight is measured in kg. Heavy black lines indicate average measurements; lines moving outward indicate one, two and three standard deviations from the mean.

Supplemental Tables

Supplemental Table S1. Full table of clinical feature of *POGZ* individuals.

See accompanying Excel spreadsheet.

Supplemental Table S2. *POGZ* MIP pool design and quality measures.

See accompanying Excel spreadsheet.

Supplemental Table S3. Summary of known *POGZ* mutations.

See accompanying Excel spreadsheet.

Supplemental Table S4. Clinical features of *de novo* missense carriers.

Individual	**EE10**	**EE11**	**EE12**	**EE13**
Mutation	c.2396G>A (p.Ser799Asn)	c.941G>A (p.Ser314Asn)	c.1121G>A (p.Arg374Gln)	c.1790A>G (p.Tyr597Cys)
Age (years)	3	9	4	12
Gender	M	M	F	M
ID/DD	?	+	-	- (very high IQ)
Speech/language development	Delayed	Delayed; primarily non-verbal	Normal	Normal
Motor development	?	?	Normal	Below average fine motor coordination
Behavior/ ***ASD***	Restricted, repetitive +	Anxiety, aggression, attention deficit, poor eye contact, repetitive behaviors +	Normal -	Limited gesturing, poor eye contact, anxiety, depression +
Microcephaly	?	-	- (very large head +2.28 SD)	-
Feeding problems	?	?	?	?
Vision problems	?	+ (unspecified)	-	?
Obesity tendency	?	Above average height, weight, and hcz	Above average (+1.54 SD)	Above average (+1.172 SD)
Other	N/A	Lactose intolerance, sleep disturbance, behaviors improve with fever	N/A	Very tall (+3.07 SD)
Additional *de novo* variants of clinical interest	N/A	N/A	N/A	N/A

ID/DD: intellectual disability/developmental delay; ASD: autism spectrum disorder (+: formal diagnosis)

Supplemental Table S5. Inherited *POGZ* events.

Chr	Pos	Ref	Alt	Individual	Cohort	Accession	Function	CADD	Inheritance
1	151381250	C	C/T	219-2320-0001	TASC	NM_001194937.1	missense	33	paternal
1	151395941	C	C/T	03C16571	AGRE	NM_001194937.1	missense	35	maternal

Supplemental Table S6. Statistical analysis of clinical observations in *POGZ* individuals with an ASD diagnosis.

Phenotype	*POGZ* sample	SSC Cohort	Statistic
Feeding Problems	4 of 8	508 of 2724	Fisher's Exact (2-tailed) p = 0.045
Sleeping Problems	3 of 4	1979 of 2757	Fisher's Exact (2-tailed) p = 0.100
Vision Problems	8 of 10	534 of 2743	Fisher's Exact (2-tailed) p= 0.000067
Hyperactivity	5 of 7	515 of 2718	Fisher's Exact (2-tailed) p = 0.004
Obesity	4 of 10	464 of 2757	Fisher's Exact (2-tailed) p = 0.07
Microcephaly	3 of 12	71 of 2757	Fisher's Exact (2-tailed) p = 0.003

Sample sizes for each comparison differed as a function of available data for each phenotype. To account for multiple comparisons, a Bonferroni-corrected p-value of 0.008 was used to establish significance.

Supplemental Table S7. Bonferroni-corrected permutation test p-values between GTEx brain and pituitary tissues for POGZ isoform 2 (NM_207171.2).

	Amygdala	Anterior cingulate cortex	Caudate	Cerebellar Hemisphere	Cerebellum	Cortex	Frontal Cortex	Hippo-campus	Hypoth-alamus	Nucleus accumbens	Putamen	Spinal cord	Substantia nigra
Anterior cingulate cortex	40.982												
Caudate	2.457	2.439											
Cerebellar Hemisphere	0.009	0.009	0.009										
Cerebellum	0.009	0.009	0.009	7.552									
Cortex	0.009	0.009	0.009	0.009	0.009								
Frontal Cortex	8.071	8.799	28.471	0.009	0.009	0.009							
Hippocampus	22.129	24.240	12.047	0.009	0.009	0.009	24.422						
Hypothalamus	15.205	16.660	20.637	0.009	0.009	0.009	36.187	34.267					
Nucleus accumbens	0.892	0.892	17.134	0.009	0.009	0.237	10.391	4.104	7.680				
Putamen	37.634	30.800	0.828	0.009	0.009	0.009	3.767	13.794	8.653	0.237			
Spinal cord	0.018	0.009	0.027	0.009	0.009	40.473	0.027	0.009	0.009	0.692	0.009		
Substantia nigra	11.383	11.583	36.105	0.009	0.009	0.091	42.202	25.705	34.604	17.780	6.196	0.200	
Pituitary	0.009	0.009	0.009	0.209	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009

Supplemental Table S8. Bonferroni-corrected permutation test p-values between GTEx brain and pituitary tissues for POGZ isoform 3 (NM_145796.3).

	Amygdala	Anterior cingulate cortex	Caudate	Cerebellar Hemisphere	Cerebellum	Cortex	Frontal Cortex	Hippo-campus	Hypoth-alamus	Nucleus accumbens	Putamen	Spinal cord	Substantia nigra
Anterior cingulate cortex	16.524												
Caudate	0.173	1.001											
Cerebellar Hemisphere	0.009	0.009	0.009										
Cerebellum	0.009	0.009	0.009	21.692									
Cortex	0.009	0.009	0.009	0.009	0.009								
Frontal Cortex	0.009	0.009	0.437	0.009	0.009	0.009							
Hippocampus	7.179	23.230	7.643	0.009	0.009	0.009	0.036						
Hypothalamus	0.055	1.001	42.138	0.009	0.009	0.009	0.773	7.115					
Nucleus accumbens	0.036	0.291	27.871	0.009	0.009	0.009	1.520	3.294	32.038				
Putamen	14.222	41.055	1.465	0.009	0.009	0.009	0.009	26.906	1.456	0.482			
Spinal cord	0.018	0.009	4.440	0.009	0.009	0.009	24.258	0.382	5.232	9.408	0.055		
Substantia nigra	3.066	11.074	22.293	0.009	0.009	0.009	0.428	28.562	20.892	12.557	14.149	2.375	
Pituitary	0.009	0.009	0.009	0.364	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009	0.009

Supplemental Table S9. Quantitative PCR of *row* expression in an inducible *Drosophila* system.

Sample			Reference Gene = PolII 31164+31165													
			GOI				Reference Gene									
			Well	Ct	Ctav	Sd	Well	Ct	Ctav	Sd	dCT	Avr dCT	Sd	ddCT	rel gene expr	T-test
row kd (6165xActin)	**1**	**1**	A1	28.68754	28.614	0.103	E1	26.26325	26.303	0.056	2.311	2.450	0.130	2.025	24.57	0.0003
	1	**1**	A2	28.5412			E2	26.34277								
	2	**2**	A3	28.72045	28.805	0.119	E3	26.41791	26.334	0.119	2.471					
	2	**2**	A4	28.88879			E4	26.24986								
	3	**3**	A5	28.37615	28.181	0.275	E5	25.69519	25.613	0.117	2.569					
	3	**3**	A6	27.98654			E6	25.52997								
Control (W1118)	**1**	**c1**	B1	26.96691	27.054	0.122	F1	27.00628	26.863	0.203	0.191	0.426	0.264			
	1	**c1**	B2	27.14013			F2	26.71957								
	2	**c2**	B3	26.80542	26.710	0.135	F3	26.00489	25.999		0.711					
	2	**c2**	B4	26.6146			F4	25.99265								
	3	**c3**	B5	26.48142	26.385	0.136	F5	26.01116	26.010	0.001	0.375					
	3	**c3**	B6	26.28842			F6	26.00912								

Drosophila larvae RNA was isolated in three biological replicates from 3rd instar larvae using RNeasy Lipid Tissue Mini Kit (Qiagen) and treated with DNase (DNAfree Kit, Ambion). First-strand cDNA synthesis was performed using the iScript cDNA Synthesis Kit (Biorad). Gene expression was analyzed by real-time PCR (7900HT Fast Real-Time PCR system, Applied Biosystems). PCR reactions were performed in a volume of 25 µl containing 150 nM primers and GoTaq Green Mastermix (Promega). Primer sequences used for amplification of *row*: 5'-CCTTTAAGGGCAAAGTGCTG-3' and 5'-ACTCCAGGTAGGCGATGTTG-3'. *PolII* was used as reference gene, primer sequences: 5'-TCAGAGTCCGCGTAACACC-3-3', 5'- TGGTCACAAGTGGCTTCATC-3'.

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