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

Holly A.F. Stessman,[1,28] Marjolein H. Willemsen,[2,3,28,*] Michaela Fenckova,[2,3] Osnat Penn,[1] Alexander Hoischen,[2,3] Bo Xiong,[1] Tianyun Wang,[4] Kendra Hoekzema,[1] Laura Vives,[1] Ida Vogel,[5] Han G. Brunner,[2,3,6] Ineke van der Burgt,[2] Charlotte W. Ockeloen,[2] Janneke H. Schuurs-Hoeijmakers,[2] Jolien S. Klein Wassink-Ruiter,[7] Connie Stumpel,[6] Servi J.C. Stevens,[6] Hans S. Vles,[8] Carlo M. Marcelis,[2] Hans van Bokhoven,[2,3] Vincent Cantagrel,[9,10] Laurence Colleaux,[9,10] Michael Nicouleau,[9,10] Stanislas Lyonnet,[9,10,11] Raphael A. Bernier,[12] Jennifer Gerdts,[12] Bradley P. Coe,[1] Corrado Romano,[13] Antonino Alberti,[13] Lucia Grillo,[14] Carmela Scuderi,[15] Magnus Nordenskjöld,[16] Malin Kvarnung,[16] Hui Guo,[4] Kun Xia,[4] Amélie Piton,[17,18] Bénédicte Gerard,[18] David Genevieve,[19] Bruno Delobel,[20] Daphne Lehalle,[21] Laurence Perrin,[22] Fabienne Prieur,[23] Julien Thevenon,[21] Jozef Gecz,[24] Marie Shaw,[24] Rolph Pfundt,[2] Boris Keren,[25,26] Aurelia Jacquette,[25] Annette Schenck,[2,3] Evan E. Eichler,[1,27,29,*] and Tjitske Kleefstra[2,3,29]

Intellectual disability (ID) and autism spectrum disorders (ASD) are genetically heterogeneous, and a significant number of genes have been associated with both conditions. A few mutations in *POGZ* have been reported in recent exome studies; however, these studies do not provide detailed clinical information. We collected the clinical and molecular data of 25 individuals with disruptive mutations in *POGZ* by diagnostic whole-exome, whole-genome, or targeted sequencing of 5,223 individuals with neurodevelopmental disorders (ID primarily) or by targeted resequencing of this locus in 12,041 individuals with ASD and/or ID. The rarity of disruptive mutations among unaffected individuals (2/49,401) highlights the significance ($p = 4.19 \times 10^{-13}$; odds ratio = 35.8) and penetrance (65.9%) of this genetic subtype with respect to ASD and ID. By studying the entire cohort, we defined common phenotypic features of *POGZ* individuals, including variable levels of developmental delay (DD) and more severe speech and language delay in comparison to the severity of motor delay and coordination issues. We also identified significant associations with vision problems, microcephaly, hyperactivity, a tendency to obesity, and feeding difficulties. Some features might be explained by the high expression of *POGZ*, particularly in the cerebellum and pituitary, early in fetal brain development. We conducted parallel studies in *Drosophila* by inducing conditional knockdown of the *POGZ* ortholog *row,* further confirming that dosage of *POGZ*, specifically in neurons, is essential for normal learning in a habituation paradigm. Combined, the data underscore the pathogenicity of loss-of-function mutations in *POGZ* and define a *POGZ*-related phenotype enriched in specific features.

Introduction

Intellectual disability (ID) and autism spectrum disorders (ASD [MIM: 209850]) are frequent, yet extremely heterogeneous, disorders. It is estimated that 10%–40% of persons with ID have ASD as a comorbidity.[1–5] Although ID and ASD often present jointly in the same individual, genetic studies searching for rare causative variants have been

[1]Department of Genome Sciences, University of Washington, Seattle, WA 98105, USA; [2]Department of Human Genetics, Radboud University Medical Center, 6500 HB Nijmegen, the Netherlands; [3]Donders Institute for Brain, Cognition and Behaviour, Radboud University Medical Center, 6500 HB Nijmegen, the Netherlands; [4]The State Key Laboratory of Medical Genetics, School of Life Sciences, Central South University, Changsha, Hunan 410078, China; [5]Department of Clinical Genetics, Aarhus University Hospital, Skejby, 8000 Aarhus, Denmark; [6]Department of Clinical Genetics, Maastricht University Medical Center+, 6229 HX Maastricht, the Netherlands; [7]Department of Genetics, University of Groningen and University Medical Center Groningen, 9700 RB Groningen, the Netherlands; [8]Department of Neurologie, Maastricht University Medical Center+, 6229 HX Maastricht, the Netherlands; [9]INSERM UMR 1163, Laboratory of Molecular and Pathophysiological Bases of Cognitive Disorders, Imagine Institute, 75015 Paris, France; [10]Paris Descartes University – Sorbonne Paris Cité, Imagine Institute, 75015 Paris, France; [11]Departments of Genetics, Hôpital Necker – Enfants Malades, Assistance Publique – Hôpitaux de Paris, 75015 Paris, France; [12]Department of Psychiatry and Behavioral Sciences, University of Washington, Seattle, WA 98105, USA; [13]Pediatrics & Medical Genetics, Istituto di Ricovero e Cura a Carattere Scientifico Associazione Oasi Maria Santissima, 94018 Troina, Italy; [14]Laboratory of Medical Genetics, Istituto di Ricovero e Cura a Carattere Scientifico Associazione Oasi Maria Santissima, 94018 Troina, Italy; [15]Neuromuscular Diseases Unit, Istituto di Ricovero e Cura a Carattere Scientifico Associazione Oasi Maria Santissima, 94018 Troina, Italy; [16]Department of Molecular Medicine and Surgery, Center for Molecular Medicine, Karolinska Institutet, 171 77 Solna, Sweden; [17]Department of Translational Medicine and Neurogenetics, Institut Génétique Biologie Moléculaire Cellulaire, 67400 Illkirch, France; [18]Laboratoire de Diagnostic Génétique, Hôpitaux Universitaires de Strasbourg, 67000 Strasbourg, France; [19]Département de Génétique Médicale, Centre de Référence Maladies Rares Anomalies du Développement et Syndromes Malformatifs Sud-Languedoc Roussillon, Hôpital Arnaud de Villeneuve, 34090 Montpellier, France; [20]Centre de Génétique Chromosomique, Hôpital Saint Vincent de Paul, Groupement des Hôpitaux de l'Institut Catholique de Lille, 59000 Lille, France; [21]Équipe EA4271-GAD, Centre de Référence Anomalies du Développement et Syndromes Malformatifs de l'Est, Fédération Hospitalo-Universitaire TRANSLAD, 21079 Dijon, France; [22]Service de Génétique Clinique, Hôpital Robert Debré, 75019 Paris, France; [23]Service de Génétique Clinique Chromosomique et Moléculaire, Centre Hospitalier Universitaire de Saint-Étienne, 42100 Saint-Étienne, France; [24]Robinson Research Institute, University of Adelaide and Women's and Children's Hospital, North Adelaide SA 5006, Australia; [25]Département de Génétique, Hôpital de la Pitié-Salpêtrière, Assistance Publique – Hôpitaux de Paris, 75013 Paris, France; [26]INSERM UM75 and U1127, CNRS UMR7225, Université Pierre et Marie Curie (Paris 6) and Institut du Cerveau et de la Moelle épinière, 75013 Paris, France; [27]Howard Hughes Medical Institute, Seattle, WA 98195, USA
[28]These authors contributed equally to this work
[29]These authors contributed equally to this work
*Correspondence: marjolein.willemsen@radboudumc.nl (M.H.W.), eee@gs.washington.edu (E.E.E.)
http://dx.doi.org/10.1016/j.ajhg.2016.02.004.

Table 1. Clinical Features of Individuals Harboring *POGZ* Mutations from ID and/or DD Cohorts

	UMCN1	UMCN2	UMCN3	UMCN4	UMCN5	UMCN6	UMCN7	UMCN8	UMCN9
Mutation	c.2590C>T (p.Arg864*)	c.3001C>T (p.Arg1001*)	c.3456_3457del (p.Glu1154 Thrfs*4)	c.2263del (p.Glu755 Serfs*36)	c.1152dup (p.Arg385 Serfs*4)	c.2432+ 1G>A (p.?)	c.2020del (p.Arg674 Valfs*9)	c.3847C>T (p.Gln1283*)	c.3456_3457del (p.Glu1154 Thrfs*4)
Age (years)	5	13	9	6	2	12	5	26	8
Gender	F	M	M	M	M	M	F	M	M
ID/DD	++	++	+/−	+	++	+	+	++	+
Speech or language delay	++	++	++	+	+	++	+	++	++
Motor delay	+	+	+	+	+	+/−	+	+	+/−
ASD	+/−	+	+/−	+/−	+/−	+	+	−	−
Microcephaly	+	+	+	−	+	−	+/−	+	−
Feeding problems	ND	+	+	+	+	−	−	+	+
Vision problems	−	+	+	−	+	+	−	−	+
Obesity tendency	ND	−	−	−	+	+	−	+	+

All HGVS annotations were annotated on RefSeq transcript (GenBank: NM_015100.3). A full clinical description for each individual can be found in Table S1. Abbreviations are as follows: mo., months; ID, intellectual disability; DD, developmental delay; ASD, autism spectrum disorder; M, male; F, female; +, formal diagnosis (mild or moderate); ++, severe presentation; +/−, possessing some features and/or mild presentation; −, not present; ND, no data.
[a]inheritance unknown.

focused on cohorts of individuals with either ID or ASD as the main inclusion criteria. At present, more than 700 genes have been implicated across a wide variety of ID syndromes with diverse clinical presentations,[6] and the total number of ID-associated genes is estimated to be around 2,000.[2] Furthermore, over 100 genes have been reported in syndromic and non-syndromic forms of ASD.[7] Not unexpectedly, many of the same genes underlie both ID and ASD, suggesting that the molecular networks and pathways in these disorders are largely overlapping.[1] Therefore, similar genetic defects might lead to either ID or ASD in different individuals or to both conditions in the same person.

The identification of candidate ID- and ASD-associated genes has increased tremendously by the widespread application of next-generation sequencing techniques, including trio-based whole-exome and whole-genome sequencing in research and routine clinical diagnostics.[8–12] Due to the extensive genetic heterogeneity, interpretation of pathogenicity of gene mutations is challenging because establishment of a conclusive molecular diagnosis is highly dependent on the identification of mutations in the same candidate gene in individuals with similar phenotypes. This is often hampered by the rarity of each individual genetic cause of ID and/or ASD. In addition, even genetic defects in the same gene can be associated with variable clinical features. To gain insight into the clinical spectrum of ID and/or ASD phenotypes associated with genes that have not been previously implicated in these conditions, it is important to collect clinical and molecular data from additional individuals.[13,14] This can be facilitated by national and international collaboration and data sharing of clinical and molecular databases. Bundling of knowledge and expertise on rare genetic causes of ID and/or ASD is of great value in the counseling of individuals. Recently, the core phenotypes of several distinct syndromes with both ASD and ID as hallmark features have been reported.[15–18] Here, we report the core phenotype of a ID and/or ASD syndrome or phenotypic gestalt, which is caused by disruption of the pogo transposable element with zinc finger domain *(POGZ* [MIM: 614787]). A small number of mutations in *POGZ* were previously reported in different cohorts of individuals with ASD, ID, or schizophrenia (MIM: 181500); however, most of these studies did not provide further detailed clinical, molecular, or functional information on these individuals, leaving the pathogenicity of these mutations unclear.[12,19–27] Meta-analyses of both small de novo deletions and/or single-nucleotide variants in families affected by ASD and developmental delay (DD) have shown that *POGZ* is likely a risk gene for neurodevelopmental disorders.[25,28] Recently, truncating mutations in *POGZ* have been reported among small numbers of unrelated individuals. Although the phenotypes were variable, there is evidence of shared phenotypic features, suggesting that mutations in this gene might represent a distinct ASD and/or ID syndrome.[29,30]

POGZ encodes a heterochromatin protein 1 α-binding protein containing a cluster of multiple C2H2-type zinc fingers, a centromere protein (CENP) B-like DNA-binding domain, and a DDE domain that might regulate gene expression.[20] *POGZ* is involved in mitosis[31] and is expressed in the human fetal and adult brain (BrainSpan Atlas and GTEx). It is hypothesized to function as a

UMCN10	EE3	EE4	FR1	FR2	FR3	FR4	FR5	FR6	EE9[a]
c.3040C>T (p.(Gln1014*))	c.2196_2198 delAG (p.Val733del)	c.2020del (p.Arg674 Valfs*9)	c.2545+1del (p.?)	c.2400dup (p.Lys801 Glnfs*7)	c.2836del (p.Asp946 Metfs*12)	c.2574del (p.His858 Glnfs*13)	c.1810G>T (p.Glu604*)	c.3001C>T (p.Arg1001*)	c.2501del (p.Leu834 Trpfs*20)
11	19	12	4	11	6	6	24	17	11 mo.
F	M	M	M	F	F	F	M	M	M
+	+	+	++	+	++	++	+	++	+/−
++	+	+	++	+	+	++	++	+	ND
+/−	++	+	++	+	+	+	+/−	+	ND
−	−	−	+	−	+	+	ND	+	ND
−	−	−	+	−	+	+	−	−	ND
+	ND	−	+	−	+	ND	−	−	ND
−	+	+	+	−	+	+	ND	+	ND
+	+	−	−	+	−	−	+	−	ND

transcriptional regulator in molecular networks crucial for neuronal function.[11,19] *POGZ* has been shown to be co-expressed with ASD- and ID-associated genes involved in chromatin remodeling and gene transcription,[32] such as *CHAMP1* (MIM: 616327).[33]

We collected a cohort of 24 individuals with de novo mutations in *POGZ* by different approaches, including whole-exome sequencing in diagnostic and research cohorts, whole-genome sequencing in a research cohort, and targeted resequencing in research cohorts. The cohorts consisted of individuals diagnosed with various neurodevelopmental disorders, including ID and/or ASD. By studying their phenotype, we were able to further define and establish the core phenotype associated with disruptive mutations in *POGZ*. We provide further support for the importance of *POGZ* in cognitive function by utilizing a *Drosophila* knockdown model of the *POGZ* ortholog *row*. We found that downregulation of *row* expression, specifically in neurons, leads to deficits in habituation, a form of non-associative learning that is highly relevant for both ID and ASD.

Materials and Methods

Individual Selection

Persons with severe *POGZ* mutations (frameshift, nonsense, and splice) were identified by different strategies. 12 individuals with unexplained ID (UMCN1, UMCN3–UMCN10, FR1, FR3, and FR6), out of approximately 2,413 screened, were identified by diagnostic exome sequencing[8] (Tables 1 and Table S1). One individual (UMCN2) was ascertained and published previously from whole-genome sequencing of 50 individuals with unexplained ID.[12] Two individuals (EE1 and EE2) were identified and published previously from research exome sequencing of 2,377 simplex families affected by ASD.[10] The remaining ten individuals were identified by a variety of targeted sequencing techniques. One individual (FR5) was identified with the Illumina TruSight One panel (Tables 1 and Table S1) from a cohort of 175 individuals with neurodevelopmental disorders. Two individuals (FR2 and FR4) were identified with a custom SureSelect panel of 275 ID-related genes (Tables 1 and Table S1) from a total of 208 probands with mild to severe ID. The remaining seven individuals (EE3–EE9) were identified with molecular inversion probe (MIP)-based sequencing (see below). Four missense events reported were identified by either research exome sequencing (in individuals EE11–EE13, reported previously)[10,27] or MIP-based sequencing (in individual EE10). Subsequent phenotypic follow-up was performed by clinical interview with individuals and families. All experiments carried out on these individual samples were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national), and proper informed consent was obtained. For identification of disruptive variation in unaffected individuals, the Exome Aggregation Consortium (ExAC) data were used with neuropsychiatric cases masked (early access to these data were provided by Daniel MacArthur). This dataset is now publically available for download on the ExAC portal.

Identification of *POGZ* Mutations by MIP Resequencing

Seven individuals with disruptive events and one individual with a missense event in *POGZ* were identified with MIP resequencing as previously described[34] from a research cohort of 12,041 individuals with unexplained ID and/or ASD (EE3–EE10). 4,025 sibling control individuals from the Simons Simplex Collection (SSC)[35] and The Autism Simplex Collection (TASC)[36] were also screened as unaffected individuals in this study. In total, we designed 53 small-molecule MIPs to tile across the coding regions and splice

junctions of the human *POGZ* locus. The design and concentration of each MIP probe used in the pool, as well as their individual performance, are detailed in Table S2. Secondary Sanger validation was performed on all individuals and their parents.

Calculation of De Novo Significance and Penetrance

De novo significance was calculated as previously described by Samocha et al.[37] with the R package denovolyzeR (v.3.1.0). To calculate the significance and penetrance of *POGZ* likely gene-disruptive (LGD) mutations in ID and/or DD, we utilized two control sets as a baseline: the first, representing 45,376 samples, was composed of the ExAC database with psychiatric cases removed (ExAC v.0.3) and the second was composed of the 4,025 control individuals sequenced by MIPs (see above). All significance calculations were based on the two LGD events found in this composite collection of 49,401 control individuals and the 25 LGD events found in 17,264 case individuals included in this study. Case-control significance was calculated with a standard two-tailed Fisher's exact test, and penetrance and its confidence bounds were calculated with the model described in Rosenfeld et al.,[38] specifically:

$$P(D \mid G) = \frac{P(G \mid D)\,P(D)}{P(G \mid D)\,P(D) + P(G \mid \overline{D})\,P(\overline{D})'}$$

where D = disease, G = genotype (the presence of an LGD event in *POGZ*), and $\overline{D}$ = absence of disease. The general population incidence of ID and/or DD in our cohort was assumed to match that described in Rosenfeld et al. (P(D) = 5.12%), given that our cohort composition has a similar representation of youth-onset diseases with an important genetic component with broad exclusion of chromosomal disorders.

Analysis of GTEx Data

Reads per kilobase of transcript per million mapped reads (RPKM) values were downloaded from the GTEx portal v.6 on 11 November 2015 and were used to generate boxplots for each of the *POGZ* isoforms included in GENCODE v.19 (NCBI browser GRCh37.p13). Ensembl identifiers from GENCODE were converted to RefSeq identifiers with the Ensembl portal (release 83). All RefSeq identifiers referenced here correspond to RefSeq release 73. To test the significance of the difference between the average expression of the pituitary and brain subtissues, permutation tests were conducted for every pair of subtissues. For each pair, we calculated the difference in means, *d*, and performed 10,000 permutations. In each permutation, the subtissue labels were randomly swapped and the difference in means calculated. We then calculated the average of the difference in means across all shuffles, *m*. We calculated p values as $(R + 1) / (N + 1)$ where *R* is the number of times the permuted difference in the mean is further from *m* than *d* and *N* is the number of permutations (10,000). Bonferroni's correction was used to correct for multiple testing.

Drosophila Knockdown Experiments

Fly stocks were kept on a standard *Drosophila* diet (cornmeal, sugar, and yeast) at 25°C and 45%–60% humidity at a 12 hr light-dark cycle. The inducible RNAi line against the *POGZ Drosophila* ortholog *row* (vdrc28196, no predicted off-targets) and its genetic background control line (vdrc60000) were obtained from the Vienna *Drosophila* Resource Center.[39] For the habituation experiments, RNAi was induced with the GAL4-UAS system and the panneuronal elav-Gal4 driver line w^{1118}; *2xGMR-wIR*; *elav-Gal4*, *UAS-Dicer-2*. The genetic elements in this line suppress eye color as required for the light-off jump response (*2xGMR-wIR*)[40,41] and increase RNAi efficiency (*UAS-Dicer2*), respectively.[39] Flies were reared and tested at 25°C and 70% humidity. For qPCR experiments, RNAi was induced with the ubiquitous actin-Gal4 driver line (w^{1118}; *actin-Gal4/CyOGFP*).

Drosophila Light-Off Jump Reflex Habituation Assay

The light-off jump habituation assay was performed as previously described.[42] Habituation of the startle jump response toward repeated light-off stimulus of 3- to 7-day-old individual male flies was tested in two independent 16-unit light-off jump habituation systems. 32 flies (16 flies per system) were simultaneously exposed to a series of 100 short (15 ms) light-off pulses with a 1 s inter-pulse interval. The noise amplitude of wing vibration after every jump response was recorded for 500 ms after the start of light-off pulse, and an appropriate threshold was applied to filter out the background noise. Data were collected and analyzed by a custom Labview Software (National Instruments). High initial jumping response to light-off pulse decreased with a growing number of trials, and flies were considered habituated when they failed to jump in five consecutive trials (no-jump criterion). Habituation was scored as the mean number of trials required to reach the non-jump criterion (trials to criterion [TTC]). The main effect of the genotype (mutant versus control, corrected for experimental day and system) on log-transformed TTC values was tested via linear model regression analysis (lm) with R statistical software (v.3.0.0).

Results

Identification of De Novo Mutations in *POGZ*

Through diagnostic whole-exome sequencing or targeted exome sequencing, we identified 15 individuals, out of approximately 2,796 case individuals, with unexplained ID and LGD mutations in *POGZ*. In order to identify additional individuals with *POGZ* mutations, we targeted and sequenced the coding portions of this gene by using MIPs (see Materials and Methods) on a large research cohort of 12,041 individuals with an ASD and/or ID diagnosis and identified seven additional events. Combined with three events that we previously identified,[10,12] we collected 19 individuals with a primary diagnosis of ID (Tables 1 and Table S1) and 6 individuals with a primary diagnosis of ASD (Table 2) with LGD mutations in *POGZ* (Figure 1 and Table S3). These include 10 nonsense, 12 frameshift, 1 in-frame deletion, and 2 splice events.

In order to determine the significance of LGD mutations at this locus in case individuals, we examined several control cohorts for similar mutations. Using the MIP-based approach,[34] we targeted and sequenced the *POGZ* locus in 4,025 unaffected sibling control individuals and identified no LGD events and one de novo missense event that has been previously published[10] (Table S3). Furthermore, when we queried the ExAC database (with neuropsychiatric cases masked), we identified two LGD events out of 45,376 individuals. Among 29,085 DD cases with published copy-number variant (CNV) data,[43] we observed

Table 2. Clinical Features of *POGZ* Individuals from ASD Cohorts

	EE1	EE2	EE5	EE6	EE7	EE8
Mutation	c.3600_3607dupTGATGACG (p.Glu1203Valfs*28)	c.3022C>T (p.Arg1008*)	c.1212C>A (p.Tyr404*)	c.538C>T (p.Gln180*)	c.3139G>T (p.Glu1047*)	c.2291del (p.Pro764Leufs*27)
Age (years)	8	14	21	7	7	6
Gender	M	M	F	F	M	F
ID/DD	+/−	+	+	+/−	+	+/−
Speech or language delay	+	+/−	+	++	++	+
Motor delay	+	++	ND	ND	+	+
ASD	+	+	+	+	+	+
Microcephaly	−	+/−	−	−	−	−
Feeding problems	−	−	ND	ND	−	+
Vision problems	+	ND	ND	ND	+	+
Obesity tendency	+	+	+/−	−	+	+

All HGVS annotations were annotated on RefSeq transcript NM_015100.3. A full clinical description for each individual can be found in Table S1.
ID, intellectual disability; DD, developmental delay; ASD, autism spectrum disorder; M, male; F, female; +, formal diagnosis (mild or moderate); ++, severe presentation; +/−, possessing some features and/or mild presentation; −, not present; ND, no data.

one 8.3 Mbp duplication that subsumed the *POGZ* locus, but no gene-breaking CNVs (Figure S1). A similar analysis of 19,584 control individuals showed no CNVs at this locus. Previously published studies reported 2 de novo LGD events among probands with schizophrenia,[21,24] 16 LGD events (including one gene-breaking CNV) among cases of ID, DD, and ASD,[20,25,26,29,30] and 1 missense event in a case of ASD;[22] however, none of these overlap directly with the 25 events reported in this study (Table S3). On the basis of these data, we estimate that protein-truncating mutations in *POGZ* are significantly enriched in ASD and/or ID individuals in comparison to the general population ($p = 4.19 \times 10^{-13}$, two-tailed Fisher's exact test; odds ratio = 35.8; 95% confidence interval [CI] = 8.9–311.9; significance calculations computed solely on the 25 cases characterized in this study). On the basis of these data, we estimate the penetrance of *POGZ* LGD mutations to be 65.9% (95% CI = 36.4%–89.8%) given the incidence of ID (5.12%) in the general population.[38]

24 of the 25 *POGZ* truncating events were shown to be de novo, and there was one individual with an LGD event for whom inheritance status could not be determined (EE9). Using a previously described statistical framework for identifying excesses of de novo mutation by gene,[37] we estimate the probability of detecting 24 or more *POGZ* de novo LGD events in 17,264 cases to be $p = 5.85 \times 10^{-39}$. In addition to these LGD events, we also identified a number of missense variants. Exome sequencing studies previously reported four de novo missense variants among families with ASD[10,22,27] (Figure 1 and Table S3). Two of these events were in probands and one was in an unaffected sibling control individual. We identified one additional de novo (Table S4) and two private, inherited (Table S5) missense events through our MIP sequencing efforts.

Clinical Description and Phenotype Analysis

The clinical features of the 25 individuals with LGD mutations in *POGZ* are summarized in Tables 1 and 2 with accompanying photographs (Figure 2). Detailed case reports are described in the Supplemental Note. Though most individuals exhibit facial dysmorphisms, there is no significant pattern that could guide to a recognizable facial gestalt. However, individuals with disruptive mutations in *POGZ* showed a clear overlap in several other clinical features. Whereas all had some degree of DD varying from borderline to severe impairment, most individuals had mild ID. Speech and language were generally classified as more severely affected than motor development. Strikingly, in several individuals, the ability to speak in sentences and write and read simple language started very late, but was eventually acquired. In addition, a distinct neurobehavioral phenotype could be recognized, including either a formal ASD diagnosis or features of ASD and, in many cases, a seemingly contrary overly social and overly friendly demeanor. Hyperactivity and sleeping problems were frequently observed as well. Other medical concerns included infections, mainly of the upper respiratory tract at a younger age, and nonspecific ocular problems. In contrast to the LGD variants, the missense variants are not clearly associated with impaired intellectual capacity. However, the missense variants seem to be associated with a behavioral phenotype, including ASD or autistic-like features, reminiscent of that seen in individuals carrying LGD variants[22] (Table S4).

To clarify the phenotype of individuals with the *POGZ* mutation, we compared several key phenotypic variables in the individuals with a *POGZ* mutation and an ASD diagnosis to those in a cohort of 2,718 children with ASD from the SSC.[35] The rate of reported problems that emerged through comprehensive individual follow-up, including

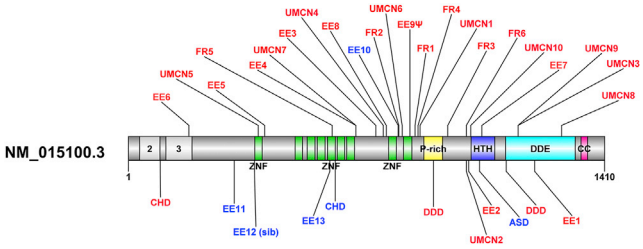


Figure 1. Protein Model of *POGZ* with Currently Identified Mutations Indicated

All mutations (indicated by individual identifiers that correspond to Tables 1 and 2 and Tables S1, S3, and S4) have been annotated on the RefSeq transcript (GenBank: NM_015100.3) (*POGZ*). Events in red are LGD and blue are missense. Mutations listed on the top of the protein structure have not been previously identified or reported. Mutations listed on the bottom of the protein structure have been published previously. Protein domains are indicated on the structure. Abbreviations are as follows: ZNF, zinc finger; HTH, helix-turn-helix; CC, coiled coil; CHD, congenital heart defect; ASD, autism spectrum disorder; DDD, developmental delay; sib, sibling. Light-gray shaded portions indicate amino acids omitted by alternatively spliced *POGZ* transcripts (3, *POGZ* isoform; 2, *POGZ* isoform 2). [Ψ]Mutations for which inheritance is unknown. All other mutations are de novo.

sleep problems, feeding problems, vision problems, hyperactivity, obesity, and microcephaly, were compared across groups with the Fisher's exact test (Table S6). It should be noted that different approaches were used to assess sleep, feeding, and vision problems in this cohort than were used in the SSC. For the individuals with a *POGZ* mutation, the presence of hyperactivity and sleep, feeding, and vision problems were identified through formal clinical assessment. For the SSC cohort, sleep, feeding, and vision problems were established through standardized medical history interview. Hyperactivity was defined as meeting clinical cut-off on parent reporting on the Child Behavior Checklist Externalizing Domain T score.[44] Obesity and microcephaly were determined through examination of standardized BMI and head circumference values. Sleep disturbance was not statistically enriched in the *POGZ* cohort. Although feeding problems and obesity showed borderline significance ($p = 0.045$ and 0.07, respectively), significant increases in vision problems ($p = 0.000067$), microcephaly ($p = 0.003$), and hyperactivity ($p = 0.004$) were observed in the individuals with a *POGZ* mutation and ASD relative to the SSC comparison cohort (Table S6).

In this case series, 12 of the 25 individuals (48%) were identified as clinically obese at some point in the developmental trajectory. This is in contrast to the feeding problems, including problems with chewing and swallowing and aversion to solid foods, that were reported in 10 of the 25 individuals (40%; Tables 1 and 2). For one individual (EE8), longitudinal growth data were available and showed that, although other growth measurements (e.g., height) were within normal ranges, body weight already exceeded three SDs above the mean starting at two-and-a-half years of age. This trend has been maintained through the age of six (the age of the last recording; Figure S2A) and is in contrast to this individual's unaffected sibling, who consumes the same diet and falls within normal body mass ranges (Figure S2B). The rate of obesity (i.e., two SDs above the mean) among children with sporadic ASD is approximately 17% (based on the SSC), in comparison to 40% in our study (calculation performed only on individuals for whom these data were available; Table S6).

POGZ Expression Analysis

We explored the normal expression patterns of *POGZ* across human tissues by using the publicly available GTEx database. Of the ten isoforms identified in GTEx, only two are highly expressed across the majority of tissues: *POGZ* isoform 3 (GenBank: NM_145796.3) and isoform 2 (GenBank: NM_207171.2) (Figures 3A and 3B). The longest annotated isoform (GenBank: NM_015100.3) (Figure 3A) does not appear to be constitutively expressed (not shown). In adult tissues, *POGZ* isoforms 2 and 3 consistently show significantly increased expression in the cerebellum and the pituitary gland ($p < 0.01$, permutation test; Tables S7 and S8) in comparison to expression in all other brain subtissues (Figure 3B). The most abundantly expressed RNA isoform 3 (GenBank: NP_665739.3) (Figure 3B, right panel) differs from the longest annotated isoform of *POGZ* only by omission of amino acids 112–189 encoded by two in-frame exons that are alternatively spliced out of the mature transcript. All individual mutations would affect protein isoform 3 of *POGZ* with the exception of that of individual EE6 (p.Gln180*; Figure 1). Isoform 2 (GenBank: NP_997054.1), which shows the second highest RNA expression pattern (Figure 3B, left panel), differs from the longest isoform by omission of amino acids 34–94. This isoform (GenBank: NM_207171.2) would contain all individual mutations (Figure 1). Further exploration of *POGZ* expression in fetal brain tissues via the BrainSpan Atlas shows that expression is highest during early embryonic development (9 post-conception weeks) and decreases gradually until birth, after which low-level expression is maintained into adulthood (Figure 3C).

Modeling *POGZ* Partial Loss of Function in *Drosophila*

To gain independent support for the implication of *POGZ* in ID/ASD pathologies and address whether the protein is required directly in neurons for normal functioning, we turned to the fruit fly *Drosophila melanogaster,* an organism that has already been successfully exploited to study specific aspects of the observed human pathologies.[46] The *Drosophila* genome harbors a single gene ortholog representing the human *POGZ* family (*POGZ*, ZNF280A-D); this ortholog is called relative of woc (*row*).[47,48] According to the ModEncode and FlyAtlas systematic expression databases,[49,50] *row* is expressed across developmental stages and tissues, and the highest expression is found in the larval CNS.[38,39] We modeled the *POGZ* loss-of-function condition by conditional knockdown of *row* in *Drosophila*

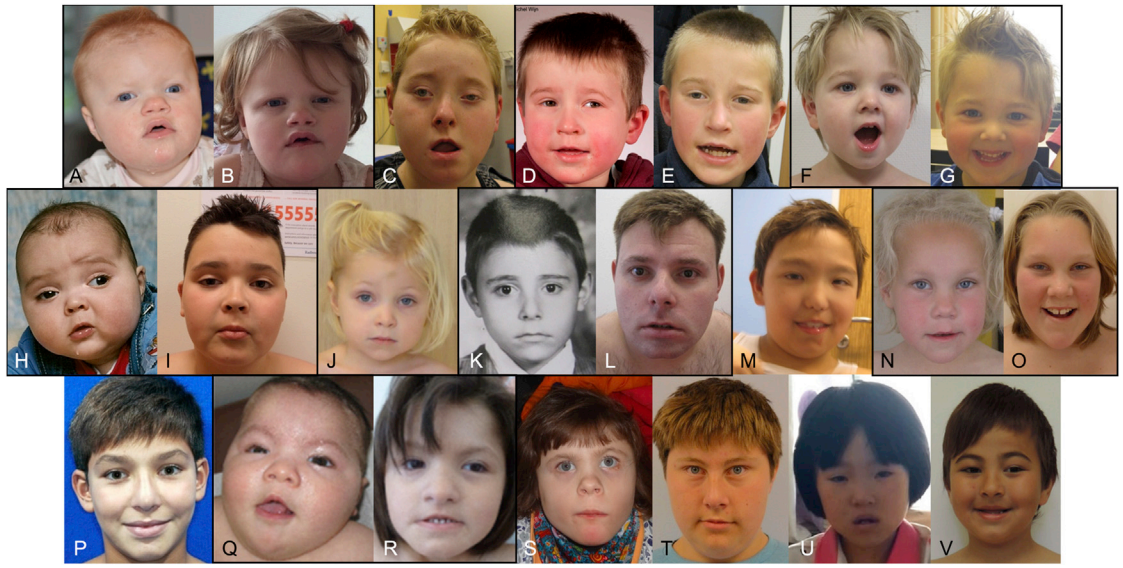


Figure 2. Clinical Photographs of Individuals with De Novo LGD Mutations in *POGZ*
Individuals harboring *POGZ* mutations show an overlap in facial features, including brachycephaly (not shown) and a broad forehead, a high nasal bridge, hypertelorism, and a thin upper lip in some. However, the facial phenotype is not very specific or recognizable.
(A and B) Individual UMCN1 at the ages of 1 year (A) and 3 years (B).
(C) Individual UMCN2 at the age of 9 years.
(D and E) Individual UMCN3 at the ages of 4 years (D) and 8 years (E).
(F and G) Individual UMCN4 at the ages of 4.5 years (F) and 5 years, 2 months (G).
(H and I) Individual UMCN6 at the ages of 6 months (H) and 11 years (I).
(J) Individual UMCN7 at the age of 4 years.
(K and L) Individual UMCN8 as a child (K) and at the age of 26 years (L).
(M) Individual UMCN9 at the age of 8 years.
(N and O) Individual UMCN10 at the ages of 4 years (N) and 11 years (O).
(P) Individual EE4 at the age of 12 years.
(Q and R) Individual FR3 at the ages of 7 months (Q) and 6 years (R).
(S) Individual FR4 at the age of 6 years.
(T) Individual EE2 at the age of 14 years.
(U) Individual EE6 at the age of 7 years.
(V) Individual EE7 at the age of 7 years.

by using the GAL4-UAS system[51] and an inducible RNAi line.[39,51] The efficacy of the *row* construct was confirmed by qRT-PCR upon ubiquitous knockdown with the *actin-Gal4* promoter line. The relative mRNA expression compared to that of the appropriate genetic background control (the promoter line crossed to the isogenic background of the RNAi line) was 25% ($p = 0.0003$; Student's t test; Table S9).

In order to assess the consequences of *row* knockdown in neurons, we crossed the inducible *row* RNAi line to the panneuronal elav-Gal4 promoter line.[40,41] The isogenic host strain of the RNAi construct crossed to the same promoter line served as genetic background controls. We assessed habituation, a non-associative form of learning and behavioral plasticity, in the light-off jump habituation paradigm as previously described.[42] In this paradigm, the strong initial reaction to a non-threatening stimulus (light-off) gradually wanes. Established *Drosophila* learning and memory mutants, as well as several *Drosophila* ID-associated gene models, have previously been shown to exhibit deficits in habituation.[40–42,52] *row*-knockdown flies, as well as their genetic background controls, showed good initial jump response (70% of flies jumping in the first five trials), indicating an absence of severe neurological defects. Whereas control flies quickly habituated to the repeatedly presented light-off stimuli (mean TTC = 10.2), the plastic behavioral response of *row* knockdown flies was strongly affected. They needed, on average, more than double the number of trials to reach the no-jump criterion ($n = 135$; mean TTC = 21.57; $p = 9.19 \times 10^{-5}$). The average jump response of *row*-knockdown and control flies, as well as the difference between mean TTC values, is depicted in Figure 4. We conclude that partial loss of function of the *POGZ* ortholog *row* in *Drosophila* neurons specifically significantly affects non-associative learning in the light-off jump reflex habituation paradigm.

Discussion

We collected a substantial cohort of 24 individuals with de novo LGD mutations in *POGZ*, enabling us to define the core phenotype. Individuals are characterized by a variable neurodevelopmental or neurobehavioral profile associated

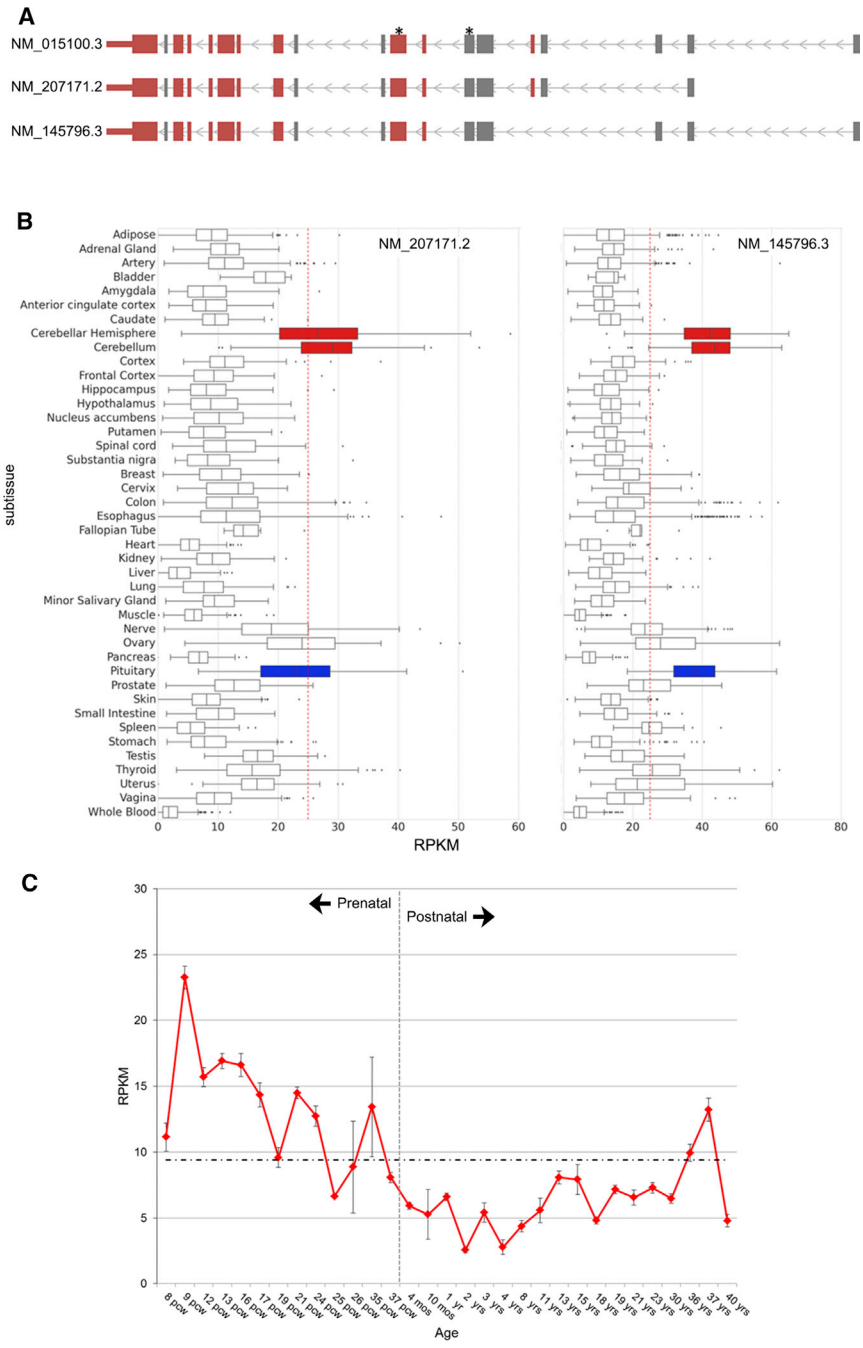


Figure 3. Expression of *POGZ* Is Highest in Cerebellum and Pituitary Tissues

(A) Gene models of three RefSeq *POGZ* isoforms. Isoform 1, the longest isoform, is shown on top (GenBank: NM_015100.3), isoform 2 in the center (GenBank: NM_207171.2), and isoform 3 on the bottom (GenBank: NM_145796.3). Exons are shown as blocks and directionality as light-gray arrows. Exons in red contain a mutation identified in this study. Black stars indicate exons in which disruptive mutations were identified in control individuals.

(B) Expression of *POGZ* shown by isoform (GenBank: NM_207171.2 on the left and GenBank: NM_145796.3 on the right), and subtissue from the GTEx database (v.6).[45] Cerebellar tissues are shown in red and the pituitary gland is shown in blue. The red dashed line indicates reads per kilobase of transcript per million mapped reads (RPKM) of 25.

(C) *POGZ* average expression of all brain subtissues across brain development (with RNA-seq RPKM values from the BrainSpan Atlas v.10; shown in red from 8 post-conception weeks to 40 years of age). Birth is indicated by a vertical gray dashed line, and the mean expression across all time points is indicated by a horizontal black dashed line.

with ID and/or either autistic features or a formal diagnosis of ASD. As expected, all individuals showed evidence of DD ranging from mild to severe. Language and speech delay presented more prominently than motor delay and coordination problems. 50% of the individuals reported here received a formal diagnosis of ASD; however, several individuals showed an atypical behavioral phenotype including overly friendly and overly social behavior and hyperactivity. Other significant phenotypic features were specific feeding difficulties that seemed to be related to oversensitivity of the oral region in several individuals, a tendency to obesity, nonspecific vision problems, and microcephaly. These clinical finding are also consistent with other recent reports of smaller cohorts of individuals with *POGZ* mutations.[29,30]

We repeated the phenotypic analysis, limiting it to those 12 individuals with a diagnosis of autism, and compared it to phenotypic data collected for 2,718 children from the SSC[35] ascertained for the presence of an ASD diagnosis. Significant increases in vision problems, hyperactivity, feeding problems, and microcephaly ($p < 0.05$) and a trend toward obesity ($p = 0.07$) were observed in the individuals with a *POGZ* mutation relative to the SSC comparison cohort (Table S6). As has been observed for other autism risk genes (*CHD8* [MIM: 610528], *DYRK1A* [MIM: 600855], and *ADNP* [MIM: 611386]), these data argue that autistic individuals harboring *POGZ* mutations represent not only a distinct genetic but also a clinical subtype of the condition defined by a gestalt of features.

It is interesting that *POGZ* was one of 21 genes recently highlighted as being recurrently mutated in individuals with congenital heart disease (CHD [MIM: 600001]) and neurodevelopmental disorders.[53] Homsy et al. considered the fact that *POGZ* mutations were also observed in cohorts of individuals with ASD and/or ID as strong evidence that CHD and neurodevelopmental disorders share a

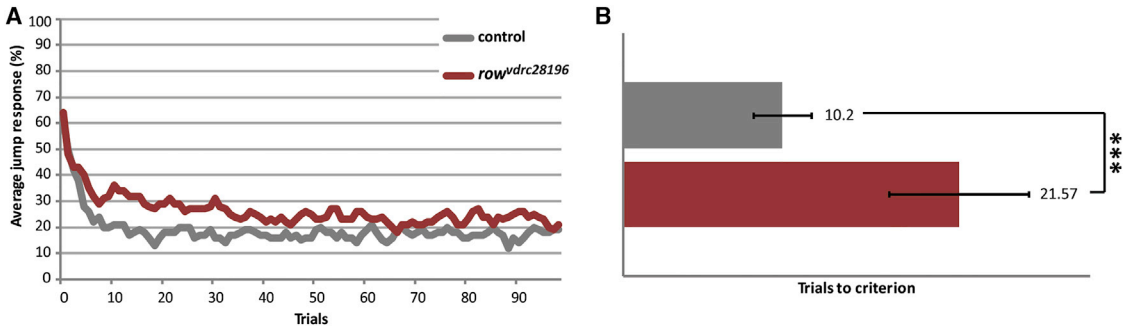


Figure 4. Knockdown of the *Drosophila POGZ* Ortholog *row* Affects Non-Associative Learning in the Light-Off Jump Reflex Habituation Paradigm

Jump responses were induced by repeated light-off pulses (100 trials) with a 1 s inter-trial interval. *row* knockdown flies ($row^{vdrc28196}$; genotype: w^{1118}; 2xGMR-wIR/+; UAS-$row^{vdrc28196}$/elav-Gal4, UAS-Dicer-2) are plotted in red, and genetic background control flies (control; genotype: w^{1118}; 2xGMR-wIR/+; elav-Gal4, UAS-Dicer-2/+) are plotted in dark gray. Linear regression model analysis revealed that flies with panneuronally-induced *row* knockdown habituated significantly slower (fold-change = 2.1;***$p < 0.001$).

(A) Average jump response (% of jumping flies) across 100 light-off trials.

(B) Mean TTC of $row^{vdrc28196}$ (TTC = 21.57, n = 135) versus mean TTC of control flies (TTC = 10.2, n = 107). Error bars indicate SEM.

common genetic etiology. Two de novo variants were identified in the CHD study, including one early splice and one missense event (Figure 1 and Table S3). In our cohort, only one of the 25 individuals (FR1) with an LGD mutation showed evidence of CHD (see Supplemental Note), and subsequent clinical follow-up of seven individuals for this phenotype provided no evidence of CHD. Interestingly, individual FR1 represented one of two splice mutations in our cohort of individuals with *POGZ* mutations. Although pleiotropic effects remain a possibility, an alternate hypothesis is that *POGZ* associates with the neurodevelopmental disease aspect and that CHD is caused by some additional risk factor in the CHD exome-sequencing-study individuals, given that many suffered from both neurodevelopmental delay and CHD.

In this study, we report three instances of the same de novo LGD mutation found in two unrelated individuals (at amino acid 674 in two individuals, at amino acid 1001 in two individuals, and at amino acid 1154 in two individuals). These events have not been reported previously in control samples or other studies.[29,30,53] In some cases (particularly at amino acid 1154), these individuals show similar phenotypes, including behavioral deficits, but at the other two sites, the pairs of individuals do not appear to be more clinically similar. These data suggest that variable expressivity overrides allelic heterogeneity with respect to phenotypic manifestation, possibly as a result of differences in the genetic background or environmental effects. Nevertheless, the identification of identical de novo events is rare among unrelated affected probands.[10] The presence of recurrent events in this study suggests that there might be a shared mechanism by which these mutations occur which will require further study to elucidate. For example, the mutation at amino acid 674 represents a CpG dinucleotide, a known hotspot for de novo mutation.[54]

We find that *POGZ* is constitutively expressed across most tissues and has significantly higher levels of expression in the cerebellum and the pituitary gland (Figure 3 and Tables S7 and S8). Also, in *Drosophila*, *row* mRNA expression is observed across most tissues, and the strongest signal is in the CNS. The cerebellum is known to regulate motor control and some cognitive functions and has been implicated in the biology of ASD as well as related conditions, such as attention deficit hyperactive disorder (ADHD [MIM: 143465]).[55] Among individuals with *POGZ* mutations, we observed marked language deficits, delayed motor development, and lack of coordination, as well as hyperactivity in some individuals, consistent with the intimate association of the cerebellum and cerebral cortex with respect to ASD pathology.[56] The higher level of expression in the pituitary also warrants further investigation in light of the findings of obesity, feeding problems, and vision deficits in these individuals. Hypothalamic-pituitary structural lesions in pediatric individuals, for example, are strongly associated with endocrine dysfunction, neuro-opthalmic presentation, and abnormal BMI and growth velocity.[57] Notably, we did not observe any individual in whom both feeding problems and obesity were present concurrently (Tables 1 and 2). Considering the potential importance of the pituitary, a future area of investigation might be to measure growth hormone production in individuals with *POGZ* mutations.

It is interesting that many of the potentially pathogenic mutations fall within the second half of the protein, suggesting that the protein domains located here might be responsible for some of the shared phenotypic features. Although little is known about the function of *POGZ* in vivo, two functional domains have been reported in the literature to lie within this region. The HP1-binding zinc-finger-like motif (amino acids 791–850) is thought to be integral for the binding of *POGZ* to heterochromatin protein 1 (CBX5, or HP1) for proper mitotic progression.[31] When *POGZ* is knocked down in human cell lines, proper cell growth and division is abrogated.[31] A second protein domain, the transposase-derived DDE domain (amino

acids 1117–1323) near the C-terminal end of the protein, is known to interact directly with PSIP1 (a.k.a., LEDGF, or p75),[58] which is known to have human expression in the fetal brain and is thought to be a transcriptional co-activator involved in neurogenesis.[59] Seizures were also a comorbidity reported in three individuals with *POGZ* mutations. These cases represent three of the four earliest truncating events in the protein that would effectively lack all of the putative functional domains.

In addition, we studied the effect of *POGZ* loss of function in a *Drosophila* model. Although human *POGZ* and *Drosophila row* share low overall sequence conservation at the protein level (10% identity), the central zinc finger motifs are well conserved (25% identity). Given the evolutionary divergence between vertebrates and invertebrates, such divergence is not unexpected among orthologs but does limit functional extrapolations given that these differences could have had effects on protein function. The proteins fulfill the reciprocal best BLAST hit criterion and are annotated orthologs on the Ensembl and Treefam databases. Moreover, human *POGZ* and *Drosophila row* show conserved protein interactions, such as CBX5/HP1,[31] ZMYM4/Wok,[60] and NFX1/Nfx2[61] (human/*Drosophila* protein symbol). Targeting *Drosophila row* revealed defects in habituation, a form of non-associative learning. This learning defect is not only relevant to the human ID phenotype of individuals with *POGZ* mutations, but also interesting in light of the co-occurring ASD. Habituation has been recently proposed as a potential mechanism underlying deficits in predictive coding in ASD, and the ability to suppress the response to known irrelevant sensory stimuli might be required for the prevention of overstimulation and salience in ASD individuals.[62]

In summary, we conclude that *POGZ* adds to the large number of proteins implicated in ID and ASD etiology present in protein complexes that act by modifying chromatin structure and gene regulation.[32] The phenotypic similarities of the individuals also predict a clinical subtype of ASD and/or ID distinct from that of individuals more generally diagnosed with autism. The enrichment of specific features but variability of phenotypic presentation is reminiscent of other mutations, especially large CNVs, associated with ASD and/or ID.[4] We estimate that de novo LGD mutations of *POGZ* might describe up to 0.14% of individuals with undefined ASD and/or ID. Based on our ascertainment, we conclude that the frequency of de novo LGD mutations is 3- to 4-fold higher in ID and/or DD than in ASD, which might explain some of the atypical ASD cases observed in this study. Indeed, ID is a comorbidity often associated with ASD (present in 31% of the SSC individuals);[35] all of the individuals with *POGZ* mutations in this study showed borderline-moderate ID, even those recruited on a primary ASD diagnosis (Table 2). Further identification of individuals with disruptive de novo mutations in *POGZ* will be required to determine whether mutations in this gene also lead to ASD in the range of normal IQ.

Supplemental Data

Supplemental Data include Acknowledgments, a Supplemental Note, two figures, and nine tables and can be found with this article online at http://dx.doi.org/10.1016/j.ajhg.2016.02.004.

Conflicts of Interest

E.E.E. is on the scientific advisory board of DNAnexus and is a consultant for Kunming University of Science and Technology as part of the 1000 China Talent Program.

Received: December 11, 2015
Accepted: February 5, 2016
Published: March 3, 2016

Web Resources

The URLs for data presented herein are as follows:

BrainSpan Atlas of the Developing Human Brain, http://www.brainspan.org/
Ensembl, http://uswest.ensembl.org/index.html/
ExAC Browser, http://exac.broadinstitute.org/
GTEx Portal, http://www.gtexportal.org/home/
Human Protein Reference Database, http://www.hprd.org/
NCBI Gene, http://www.ncbi.nlm.nih.gov/gene
NHLBI Exome Sequencing Project (ESP) Exome Variant Server, http://evs.gs.washington.edu/EVS/
OMIM, http://www.omim.org/
RefSeq, http://www.ncbi.nlm.nih.gov/RefSeq
The R Project for Statistical Computing, http://www.R-project.org/
UniProt, http://www.uniprot.org/

References

1. Srivastava, A.K., and Schwartz, C.E. (2014). Intellectual disability and autism spectrum disorders: causal genes and molecular mechanisms. Neurosci. Biobehav. Rev. *46*, 161–174.
2. van Bokhoven, H. (2011). Genetic and epigenetic networks in intellectual disabilities. Annu. Rev. Genet. *45*, 81–104.
3. Matson, J.L., Rivet, T.T., Fodstad, J.C., Dempsey, T., and Boisjoli, J.A. (2009). Examination of adaptive behavior differences in adults with autism spectrum disorders and intellectual disability. Res. Dev. Disabil. *30*, 1317–1325.
4. Mefford, H.C., Batshaw, M.L., and Hoffman, E.P. (2012). Genomics, intellectual disability, and autism. N. Engl. J. Med. *366*, 733–743.
5. Lai, M.C., Lombardo, M.V., and Baron-Cohen, S. (2014). Autism. Lancet *383*, 896–910.
6. Kochinke, K., Zweier, C., Nijhof, B., Fenckova, M., Cizek, P., Honti, F., Keerthikumar, S., Oortveld, M.A., Kleefstra, T., Kramer, J.M., et al. (2016). Systematic Phenomics Analysis Deconvolutes Genes Mutated in Intellectual Disability into Biologically Coherent Modules. Am. J. Hum. Genet. *98*, 149–164.
7. Xu, L.M., Li, J.R., Huang, Y., Zhao, M., Tang, X., and Wei, L. (2012). AutismKB: an evidence-based knowledgebase of autism genetics. Nucleic Acids Res. *40*, D1016–D1022.
8. de Ligt, J., Willemsen, M.H., van Bon, B.W., Kleefstra, T., Yntema, H.G., Kroes, T., Vulto-van Silfhout, A.T., Koolen, D.A., de Vries, P., Gilissen, C., et al. (2012). Diagnostic exome

sequencing in persons with severe intellectual disability. N. Engl. J. Med. *367*, 1921–1929.
9. Rauch, A., Wieczorek, D., Graf, E., Wieland, T., Endele, S., Schwarzmayr, T., Albrecht, B., Bartholdi, D., Beygo, J., Di Donato, N., et al. (2012). Range of genetic mutations associated with severe non-syndromic sporadic intellectual disability: an exome sequencing study. Lancet *380*, 1674–1682.
10. Iossifov, I., O'Roak, B.J., Sanders, S.J., Ronemus, M., Krumm, N., Levy, D., Stessman, H.A., Witherspoon, K.T., Vives, L., Patterson, K.E., et al. (2014). The contribution of de novo coding mutations to autism spectrum disorder. Nature *515*, 216–221.
11. De Rubeis, S., He, X., Goldberg, A.P., Poultney, C.S., Samocha, K., Cicek, A.E., Kou, Y., Liu, L., Fromer, M., Walker, S., et al.; DDD Study; Homozygosity Mapping Collaborative for Autism; UK10K Consortium (2014). Synaptic, transcriptional and chromatin genes disrupted in autism. Nature *515*, 209–215.
12. Gilissen, C., Hehir-Kwa, J.Y., Thung, D.T., van de Vorst, M., van Bon, B.W., Willemsen, M.H., Kwint, M., Janssen, I.M., Hoischen, A., Schenck, A., et al. (2014). Genome sequencing identifies major causes of severe intellectual disability. Nature *511*, 344–347.
13. Bernier, R., Golzio, C., Xiong, B., Stessman, H.A., Coe, B.P., Penn, O., Witherspoon, K., Gerdts, J., Baker, C., Vulto-van Silfhout, A.T., et al. (2014). Disruptive CHD8 mutations define a subtype of autism early in development. Cell *158*, 263–276.
14. Stessman, H.A., Bernier, R., and Eichler, E.E. (2014). A genotype-first approach to defining the subtypes of a complex disease. Cell *156*, 872–877.
15. van Bon, B.W., Coe, B.P., Bernier, R., Green, C., Gerdts, J., Witherspoon, K., Kleefstra, T., Willemsen, M.H., Kumar, R., Bosco, P., et al. (2016). Disruptive de novo mutations of DYRK1A lead to a syndromic form of autism and ID. Mol. Psychiatry *21*, 126–132.
16. Helsmoortel, C., Vulto-van Silfhout, A.T., Coe, B.P., Vandeweyer, G., Rooms, L., van den Ende, J., Schuurs-Hoeijmakers, J.H., Marcelis, C.L., Willemsen, M.H., Vissers, L.E., et al. (2014). A SWI/SNF-related autism syndrome caused by de novo mutations in ADNP. Nat. Genet. *46*, 380–384.
17. Mullegama, S.V., Alaimo, J.T., Chen, L., and Elsea, S.H. (2015). Phenotypic and molecular convergence of 2q23.1 deletion syndrome with other neurodevelopmental syndromes associated with autism spectrum disorder. Int. J. Mol. Sci. *16*, 7627–7643.
18. Giannandrea, M., Bianchi, V., Mignogna, M.L., Sirri, A., Carrabino, S., D'Elia, E., Vecellio, M., Russo, S., Cogliati, F., Larizza, L., et al. (2010). Mutations in the small GTPase gene RAB39B are responsible for X-linked mental retardation associated with autism, epilepsy, and macrocephaly. Am. J. Hum. Genet. *86*, 185–195.
19. Iossifov, I., Ronemus, M., Levy, D., Wang, Z., Hakker, I., Rosenbaum, J., Yamrom, B., Lee, Y.H., Narzisi, G., Leotta, A., et al. (2012). De novo gene disruptions in children on the autistic spectrum. Neuron *74*, 285–299.
20. Neale, B.M., Kou, Y., Liu, L., Ma'ayan, A., Samocha, K.E., Sabo, A., Lin, C.F., Stevens, C., Wang, L.S., Makarov, V., et al. (2012). Patterns and rates of exonic de novo mutations in autism spectrum disorders. Nature *485*, 242–245.
21. Fromer, M., Pocklington, A.J., Kavanagh, D.H., Williams, H.J., Dwyer, S., Gormley, P., Georgieva, L., Rees, E., Palta, P., Ruderfer, D.M., et al. (2014). De novo mutations in schizophrenia implicate synaptic networks. Nature *506*, 179–184.
22. Fukai, R., Hiraki, Y., Yofune, H., Tsurusaki, Y., Nakashima, M., Saitsu, H., Tanaka, F., Miyake, N., and Matsumoto, N. (2015). A case of autism spectrum disorder arising from a de novo missense mutation in POGZ. J. Hum. Genet. *60*, 277–279.
23. Buxbaum, J.D., Daly, M.J., Devlin, B., Lehner, T., Roeder, K., and State, M.W.; Autism Sequencing Consortium (2012). The autism sequencing consortium: large-scale, high-throughput sequencing in autism spectrum disorders. Neuron *76*, 1052–1056.
24. Gulsuner, S., Walsh, T., Watts, A.C., Lee, M.K., Thornton, A.M., Casadei, S., Rippey, C., Shahin, H., Nimgaonkar, V.L., Go, R.C., et al.; Consortium on the Genetics of Schizophrenia (COGS); PAARTNERS Study Group (2013). Spatial and temporal mapping of de novo mutations in schizophrenia to a fetal prefrontal cortical network. Cell *154*, 518–529.
25. Deciphering Developmental Disorders, S.; Deciphering Developmental Disorders Study (2015). Large-scale discovery of novel genetic causes of developmental disorders. Nature *519*, 223–228.
26. Hashimoto, R., Nakazawa, T., Tsurusaki, Y., Yasuda, Y., Nagayasu, K., Matsumura, K., Kawashima, H., Yamamori, H., Fujimoto, M., Ohi, K., et al. (2015). Whole-exome sequencing and neurite outgrowth analysis in autism spectrum disorder. J. Hum. Genet. Published online November 19, 2015. http://dx.doi.org/10.1038/jhg.2015.141.
27. Krumm, N., Turner, T.N., Baker, C., Vives, L., Mohajeri, K., Witherspoon, K., Raja, A., Coe, B.P., Stessman, H.A., He, Z.X., et al. (2015). Excess of rare, inherited truncating mutations in autism. Nat. Genet. *47*, 582–588.
28. Sanders, S.J., He, X., Willsey, A.J., Ercan-Sencicek, A.G., Samocha, K.E., Cicek, A.E., Murtha, M.T., Bal, V.H., Bishop, S.L., Dong, S., et al.; Autism Sequencing Consortium (2015). Insights into Autism Spectrum Disorder Genomic Architecture and Biology from 71 Risk Loci. Neuron *87*, 1215–1233.
29. White, J., Beck, C.R., Harel, T., Posey, J.E., Jhangiani, S.N., Tang, S., Farwell, K.D., Powis, Z., Mendelsohn, N.J., Baker, J.A., et al. (2016). POGZ truncating alleles cause syndromic intellectual disability. Genome Med. *8*, 3.
30. Ye, Y., Cho, M.T., Retterer, K., Alexander, N., Ben-Omran, T., Al-Mureikhi, M., Cristian, I., Wheeler, P., Crain, C., Zand, D., et al. (2015). De novo POGZ mutations are associated with neurodevelopmental disorders and microcephaly. Cold Spring Harb. Mol. Case Stud. *1*, a000455.
31. Nozawa, R.S., Nagao, K., Masuda, H.T., Iwasaki, O., Hirota, T., Nozaki, N., Kimura, H., and Obuse, C. (2010). Human POGZ modulates dissociation of HP1alpha from mitotic chromosome arms through Aurora B activation. Nat. Cell Biol. *12*, 719–727.
32. Hormozdiari, F., Penn, O., Borenstein, E., and Eichler, E.E. (2015). The discovery of integrated gene networks for autism and related disorders. Genome Res. *25*, 142–154.
33. Isidor, B., Kury, S., Rosenfeld, J.A., Besnard, T., Schmitt, S., Joss, S., Davies, S.J., Lebel, R.R., Henderson, A., Schaaf, C.P., et al. (2016). De novo truncating mutations in the kinetochore-microtubules attachment gene CHAMP1 cause syndromic intellectual disability. Hum. Mutat. Published online January 11, 2016. http://dx.doi.org/10.1002/humu.22952.
34. O'Roak, B.J., Stessman, H.A., Boyle, E.A., Witherspoon, K.T., Martin, B., Lee, C., Vives, L., Baker, C., Hiatt, J.B., Nickerson, D.A., et al. (2014). Recurrent de novo mutations implicate novel genes underlying simplex autism risk. Nat. Commun. *5*, 5595.
35. Fischbach, G.D., and Lord, C. (2010). The Simons Simplex Collection: a resource for identification of autism genetic risk factors. Neuron *68*, 192–195.

36. Buxbaum, J.D., Bolshakova, N., Brownfeld, J.M., Anney, R.J., Bender, P., Bernier, R., Cook, E.H., Coon, H., Cuccaro, M., Freitag, C.M., et al. (2014). The Autism Simplex Collection: an international, expertly phenotyped autism sample for genetic and phenotypic analyses. Mol. Autism *5*, 34.
37. Samocha, K.E., Robinson, E.B., Sanders, S.J., Stevens, C., Sabo, A., McGrath, L.M., Kosmicki, J.A., Rehnström, K., Mallick, S., Kirby, A., et al. (2014). A framework for the interpretation of de novo mutation in human disease. Nat. Genet. *46*, 944–950.
38. Rosenfeld, J.A., Coe, B.P., Eichler, E.E., Cuckle, H., and Shaffer, L.G. (2013). Estimates of penetrance for recurrent pathogenic copy-number variations. Genet. Med. *15*, 478–481.
39. Dietzl, G., Chen, D., Schnorrer, F., Su, K.C., Barinova, Y., Fellner, M., Gasser, B., Kinsey, K., Oppel, S., Scheiblauer, S., et al. (2007). A genome-wide transgenic RNAi library for conditional gene inactivation in Drosophila. Nature *448*, 151–156.
40. Willemsen, M.H., Nijhof, B., Fenckova, M., Nillesen, W.M., Bongers, E.M., Castells-Nobau, A., Asztalos, L., Viragh, E., van Bon, B.W., Tezel, E., et al. (2013). GATAD2B loss-of-function mutations cause a recognisable syndrome with intellectual disability and are associated with learning deficits and synaptic undergrowth in Drosophila. J. Med. Genet. *50*, 507–514.
41. van Bon, B.W., Oortveld, M.A., Nijtmans, L.G., Fenckova, M., Nijhof, B., Besseling, J., Vos, M., Kramer, J.M., de Leeuw, N., Castells-Nobau, A., et al. (2013). CEP89 is required for mitochondrial metabolism and neuronal function in man and fly. Hum. Mol. Genet. *22*, 3138–3151.
42. Kramer, J.M., Kochinke, K., Oortveld, M.A., Marks, H., Kramer, D., de Jong, E.K., Asztalos, Z., Westwood, J.T., Stunnenberg, H.G., Sokolowski, M.B., et al. (2011). Epigenetic regulation of learning and memory by Drosophila EHMT/G9a. PLoS Biol. *9*, e1000569.
43. Coe, B.P., Witherspoon, K., Rosenfeld, J.A., van Bon, B.W., Vulto-van Silfhout, A.T., Bosco, P., Friend, K.L., Baker, C., Buono, S., Vissers, L.E., et al. (2014). Refining analyses of copy number variation identifies specific genes associated with developmental delay. Nat. Genet. *46*, 1063–1071.
44. Achenbach, T.M., Howell, C.T., Quay, H.C., and Conners, C.K. (1991). National survey of problems and competencies among four- to sixteen-year-olds: parents' reports for normative and clinical samples. Monogr. Soc. Res. Child Dev. *56*, 1–131.
45. GTEx Consortium (2015). Human genomics. The Genotype-Tissue Expression (GTEx) pilot analysis: multitissue gene regulation in humans. Science *348*, 648–660.
46. van der Voet, M., Nijhof, B., Oortveld, M.A., and Schenck, A. (2014). Drosophila models of early onset cognitive disorders and their clinical applications. Neurosci. Biobehav. Rev. *46*, 326–342.
47. Flicek, P., Ahmed, I., Amode, M.R., Barrell, D., Beal, K., Brent, S., Carvalho-Silva, D., Clapham, P., Coates, G., Fairley, S., et al. (2013). Ensembl 2013. Nucleic Acids Res. *41*, D48–D55.
48. Ruan, J., Li, H., Chen, Z., Coghlan, A., Coin, L.J., Guo, Y., Hériché, J.K., Hu, Y., Kristiansen, K., Li, R., et al. (2008). TreeFam: 2008 Update. Nucleic Acids Res. *36*, D735–D740.
49. Graveley, B.R., Brooks, A.N., Carlson, J.W., Duff, M.O., Landolin, J.M., Yang, L., Artieri, C.G., van Baren, M.J., Boley, N., Booth, B.W., et al. (2011). The developmental transcriptome of Drosophila melanogaster. Nature *471*, 473–479.
50. Chintapalli, V.R., Wang, J., and Dow, J.A. (2007). Using FlyAtlas to identify better Drosophila melanogaster models of human disease. Nat. Genet. *39*, 715–720.
51. Brand, A.H., and Perrimon, N. (1993). Targeted gene expression as a means of altering cell fates and generating dominant phenotypes. Development *118*, 401–415.
52. Engel, J.E., and Wu, C.F. (1996). Altered habituation of an identified escape circuit in Drosophila memory mutants. J. Neurosci. *16*, 3486–3499.
53. Homsy, J., Zaidi, S., Shen, Y., Ware, J.S., Samocha, K.E., Karczewski, K.J., DePalma, S.R., McKean, D., Wakimoto, H., Gorham, J., et al. (2015). De novo mutations in congenital heart disease with neurodevelopmental and other congenital anomalies. Science *350*, 1262–1266.
54. Francioli, L.C., Polak, P.P., Koren, A., Menelaou, A., Chun, S., Renkens, I., van Duijn, C.M., Swertz, M., Wijmenga, C., van Ommen, G., et al.; Genome of the Netherlands Consortium (2015). Genome-wide patterns and properties of de novo mutations in humans. Nat. Genet. *47*, 822–826.
55. Gowen, E., and Miall, R.C. (2007). The cerebellum and motor dysfunction in neuropsychiatric disorders. Cerebellum *6*, 268–279.
56. D'Mello, A.M., and Stoodley, C.J. (2015). Cerebro-cerebellar circuits in autism spectrum disorder. Front. Neurosci. *9*, 408.
57. Taylor, M., Couto-Silva, A.C., Adan, L., Trivin, C., Sainte-Rose, C., Zerah, M., Valteau-Couanet, D., Doz, F., Chalumeau, M., and Brauner, R. (2012). Hypothalamic-pituitary lesions in pediatric patients: endocrine symptoms often precede neuro-ophthalmic presenting symptoms. J. Pediatr. *161*, 855–863.
58. Bartholomeeusen, K., Christ, F., Hendrix, J., Rain, J.C., Emiliani, S., Benarous, R., Debyser, Z., Gijsbers, R., and De Rijck, J. (2009). Lens epithelium-derived growth factor/p75 interacts with the transposase-derived DDE domain of PogZ. J. Biol. Chem. *284*, 11467–11477.
59. Chylack, L.T., Jr., Fu, L., Mancini, R., Martin-Rehrmann, M.D., Saunders, A.J., Konopka, G., Tian, D., Hedley-Whyte, E.T., Folkerth, R.D., and Goldstein, L.E. (2004). Lens epithelium-derived growth factor (LEDGF/p75) expression in fetal and adult human brain. Exp. Eye Res. *79*, 941–948.
60. Vermeulen, M., Eberl, H.C., Matarese, F., Marks, H., Denissov, S., Butter, F., Lee, K.K., Olsen, J.V., Hyman, A.A., Stunnenberg, H.G., and Mann, M. (2010). Quantitative interaction proteomics and genome-wide profiling of epigenetic histone marks and their readers. Cell *142*, 967–980.
61. Castello, A., Fischer, B., Eichelbaum, K., Horos, R., Beckmann, B.M., Strein, C., Davey, N.E., Humphreys, D.T., Preiss, T., Steinmetz, L.M., et al. (2012). Insights into RNA biology from an atlas of mammalian mRNA-binding proteins. Cell *149*, 1393–1406.
62. Ramaswami, M. (2014). Network plasticity in adaptive filtering and behavioral habituation. Neuron *82*, 1216–1229.

The American Journal of Human Genetics, Volume *98*

Supplemental Information

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

Holly A.F. Stessman, Marjolein H. Willemsen, Michaela Fenckova, Osnat Penn, Alexander Hoischen, Bo Xiong, Tianyun Wang, Kendra Hoekzema, Laura Vives, Ida Vogel, Han G. Brunner, Ineke van der Burgt, Charlotte Ockeloen, Janneke Schuurs-Hoeijmakers, Jolien S. Klein Wassink-Ruiter, Connie Stumpel, Servi J.C. Stevens, J. Vles, Carlo Marcelis, Hans van Bokhoven, Vincent Cantagrel, Laurence Colleaux, Michael Nicouleau, Stanislas Lyonnet, Raphael A. Bernier, Jennifer Gerdts, Bradley P. Coe, Corrado Romano, Antonino Alberti, Lucia Grillo, Carmela Scuderi, Magnus Nordenskjöld, Malin Kvarnung, Hui Guo, Kun Xia, Amélie Piton, Bénédicte Gerard, David Genevieve, Bruno Delobel, Daphne Lehalle, Laurence Perrin, Fabienne Prieur, Julien Thevenon, Jozef Gecz, Marie Shaw, Rolph Pfundt, Boris Keren, Aurelia Jacquette, Annette Schenck, Evan E. Eichler, and Tjitske Kleefstra

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.

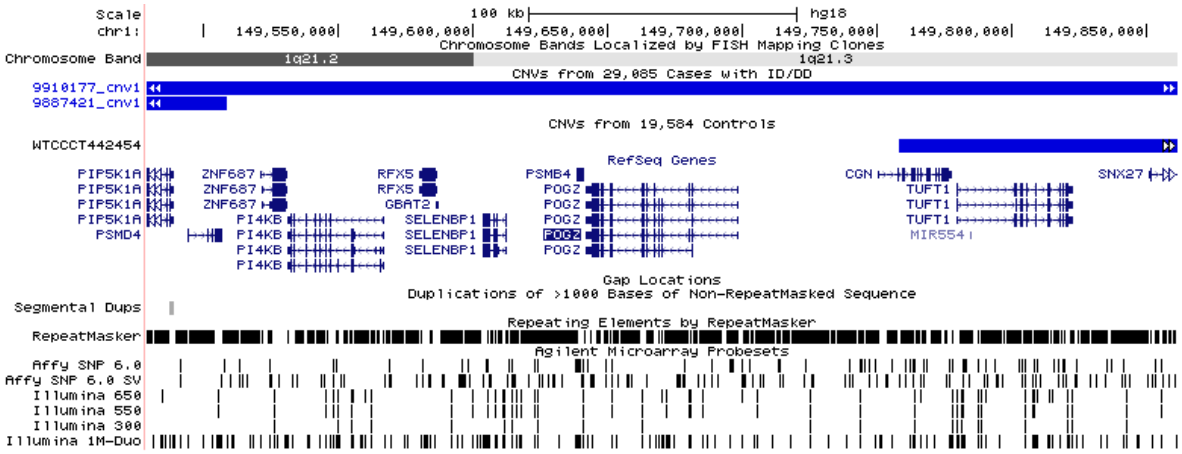
Supplemental Information

Acknowledgements

We thank the individuals and their families for participation in this study. We acknowledge the Vienna Drosophila Resource Center and Bloomington Drosophila stock center (NIH P40OD018537) and thank Daniel MacArthur for providing early access to the ExAC v0.3 database with psychiatric cases removed. This research was supported, in part, by the following: Simons Foundation Autism Research Initiative (SFARI 303241) and NIH (R01MH101221) to E.E.E., VIDI and TOP grants (917-96-346, 912-12-109) from the Netherlands Organization for Scientific Research and Horizon 2020 Marie Sklodowska-Curie European Training Network (MiND, 643051) to A.S., the Netherlands Organization for Health Research and Development, ZonMw (grant 907-00-365) to T.K., Agence de Biomedecine, Fondation Jerome Lejeune and CREGEMES to A.P., the NHGRI Interdisciplinary Training in Genome Science Grant (T32HG00035) to H.A.F.S, Australian NHMRC grants 628952 and 1041920 to J.G., the Human Frontier Science Program postdoctoral fellowship to O.P., the Region Burgundy to J.T. and D.L., and grant ANR-12-PDOC-026 to V.C. E.E.E. is an investigator of the Howard Hughes Medical Institute. We thank T. Brown for assistance in editing this manuscript. We would also like to thank Jean-Louis Mandel, Francesca Mattioli, Claire Feger, Elsa Nourisson, Laurence Faivre, Paul Kuentz, Yannis Duffourd, and Jean-François Deleuze. We are grateful to the participating Simons Simplex Collection (SSC) sites, as well as the principal investigators (A. Beaudet, R. Bernier, J. Constantino, E. Cook, E. Fombonne, D. Geschwind, R. Goin-Kochel, E. Hanson, D. Grice, A. Klin, D. Ledbetter, C. Lord, C. Martin, D. Martin, R. Maxim, J. Miles, O. Ousley, K. Pelphrey, B. Peterson, J. Piggot, C. Saulnier, M. State, W. Stone, J. Sutcliffe, C. Walsh, Z. Warren and E. Wijsman). We appreciate obtaining access to phenotypic data on Simons Foundation Autism Research Initiative (SFARI) Base. Approved researchers can obtain the SSC population data set described in this study by applying at https://base.sfari.org/.

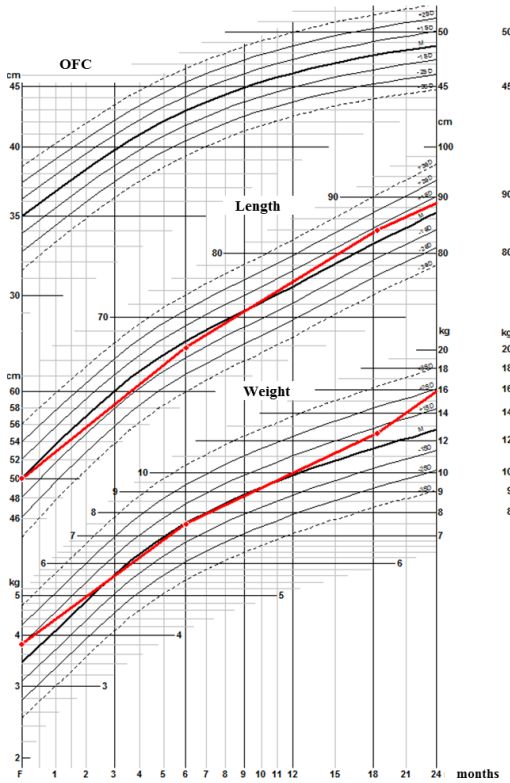
During revision of this manuscript another study reporting five unrelated ID individuals was published by White and colleagues[3]. Their descriptions of the phenotypic features are largely in agreement with our data.

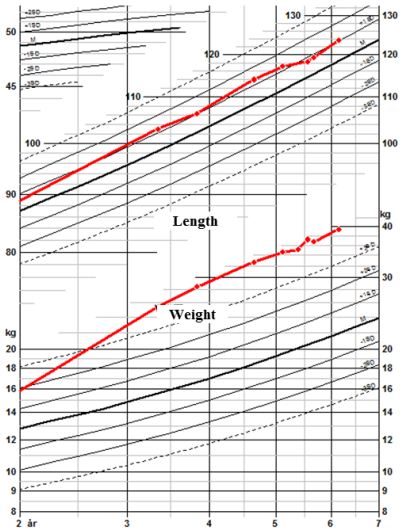
Supplemental Figures



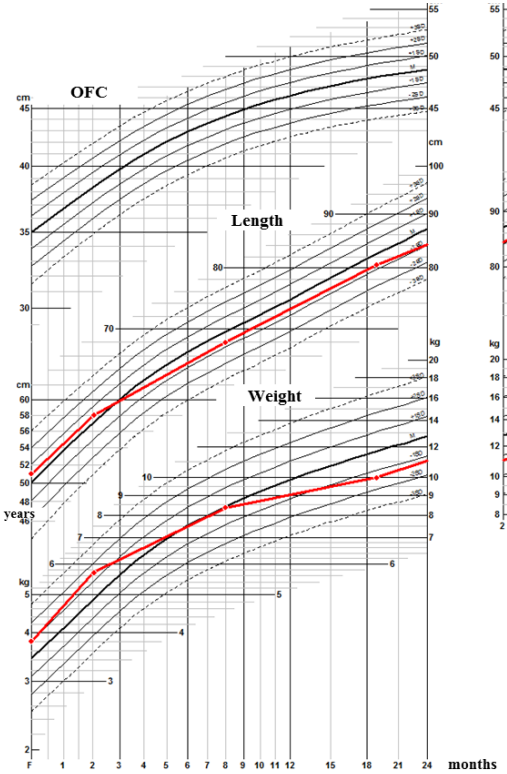
Supplemental Figure S1. CNVs intersecting *POGZ*. Examination of *POGZ* in the context of a CNV morbidity map generated from 29,085 individuals with ID/DD and 19,584 population controls identifies only a single overlapping 8.3 Mbp duplication (blue bar) in an affected individual and no events in controls[4].

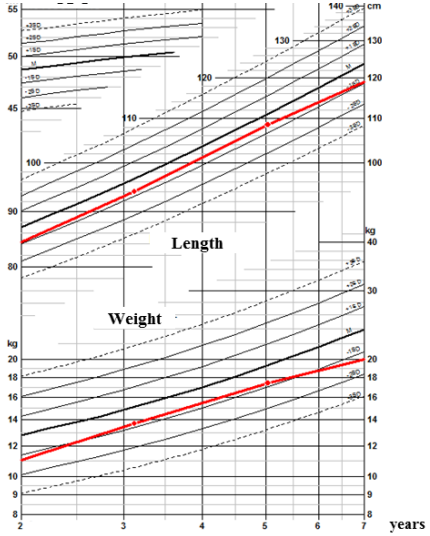
A





B





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'.

Supplemental References

1. Deprez, L., Weckhuysen, S., Peeters, K., Deconinck, T., Claeys, K.G., Claes, L.R., Suls, A., Van Dyck, T., Palmini, A., Matthijs, G., et al. (2008). Epilepsy as part of the phenotype associated with ATP1A2 mutations. Epilepsia 49, 500-508.
2. Iossifov, I., O'Roak, B.J., Sanders, S.J., Ronemus, M., Krumm, N., Levy, D., Stessman, H.A., Witherspoon, K.T., Vives, L., Patterson, K.E., et al. (2014). The contribution of de novo coding mutations to autism spectrum disorder. Nature 515, 216-221.
3. White, J., Beck, C.R., Harel, T., Posey, J.E., Jhangiani, S.N., Tang, S., Farwell, K.D., Powis, Z., Mendelsohn, N.J., Baker, J.A., et al. (2016). POGZ truncating alleles cause syndromic intellectual disability. Genome medicine 8, 3.
4. Coe, B.P., Witherspoon, K., Rosenfeld, J.A., van Bon, B.W., Vulto-van Silfhout, A.T., Bosco, P., Friend, K.L., Baker, C., Buono, S., Vissers, L.E., et al. (2014). Refining analyses of copy number variation identifies specific genes associated with developmental delay. Nature genetics 46, 1063-1071.