Pathogenic POGZ mutation causes impaired cortical development and reversible autism-like phenotypes

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POGZ transposable element derived with ZNF domain has been identified as one of the most recurrently de novo mutated genes in patients with neurodevelopmental disorders (NDDs), including autism spectrum disorder (ASD), intellectual disability and White-Sutton syndrome; however, the neurobiological basis behind these disorders remains unknown. Here, we show that POGZ regulates neuronal development and that ASD-related de novo mutations impair neuronal development in the developing mouse brain and induced pluripotent cell lines from an ASD patient. We also develop the first mouse model heterozygous for a de novo POGZ mutation identified in a patient with ASD, and we identify ASD-like abnormalities in the mice. Importantly, social deficits can be treated by compensatory inhibition of elevated cell excitability in the mice. Our results provide insight into how de novo mutations on high-confidence ASD genes lead to impaired mature cortical network function, which underlies the cellular pathogenesis of NDDs, including ASD.
Neurodevelopmental disorders (NDDs), including autism spectrum disorder (ASD) and intellectual disability (ID), are characterized by early life onset with aberrant brain development, leading to social and cognitive abnormalities that span a wide range of functions and are highly heterogeneous among individuals. Since the prevalence rate of NDDs has recently been increasing dramatically, NDDs are becoming a significant medical and social burden. Among NDDs, the prevalence rate of ASD is considerable, estimated to be ~1 in 40 children. Despite the high heritability of ASD, recent studies have suggested that the disease is highly genetically heterogeneous, with rare genetic variants as well as common variants. Accordingly, the molecular etiology of ASD and that the genetic cause is unidentified in ~90% of patients with the condition. The genetic cause is unidentified in ~90% of patients with the condition. The molecular etiology of ASD remains largely unclear. There are no pharmacological medications to treat the core symptoms of ASD; mechanism-based drug development and therapeutic strategies are imperative.

Genetic and epidemiological studies have suggested that de novo mutations, spontaneous rare mutations that appear in an affected child but not in the unaffected parents, significantly contribute to ASD and that ~3–10% of ASD risk is explained by de novo mutations in exons. Importantly, a recent, comprehensive exome analysis has identified high-confidence ASD genes. Although, among these high-confidence ASD genes, recent studies have found that haploinsufficiency of ARID1B or CHD8 causes ASD-related abnormalities in mice, further direct assessments of the biological significance of ASD-associated de novo mutations are necessary to fully understand the contribution of de novo mutations to the core features of ASD.

We and other groups have found that Pogo transposable element derived with ZNF domain (POGZ) is one of the most recurrently mutated genes in patients with NDDs, particularly ASD and ID; the number of reported mutations continues to increase (see Fig. 1a and Supplementary Table 1; we classified patients into ASD, ID, and White–Sutton syndrome according to the original diagnosis in each report). POGZ mutations are also recurrently found in patients with White–Sutton syndrome, characterized by ID and specific facial features. POGZ interacts with the SP1 domain.
transcription factor, heterochromatin protein 1 (HP1), and chromodomain helicase DNA-binding protein 4 (CHD4)\textsuperscript{35–37}, which suggests that POGZ functions as a chromatin regulator; however, the role of POGZ in brain development and the biological significance of ASD-associated de novo POGZ mutations in the etiology of ASD are largely unknown.

In this study, we developed the first mouse model that carried a pathogenic de novo mutation of POGZ identified in an ASD patient. From the same patient, we established induced pluripotent stem cell (iPSC) lines with the same de novo POGZ mutated (Q1042R) as the model mouse. Comprehensively examining these human and mouse materials, we determined that the de novo mutation in POGZ impaired the cellular localization of the POGZ protein and hindered cortical neuronal development. We also determined that this de novo mutation in POGZ caused ASD-related behavioral abnormalities and that these abnormalities were pharmacologically treatable even in adulthood. Importantly, de novo POGZ mutations identified in unaffected controls had no damaging effect on POGZ function in neuronal development. Together, these observations provide the first in vivo evidence suggesting that ASD-associated de novo mutations in a high-confidence ASD gene are critical for a wide range of processes involved in ASD pathogenesis.

Results
De novo mutations in POGZ impair its nuclear localization. POGZ has been identified as one of the most recurrently de novo-mutated genes in patients with NDDs (Fig. 1a, c, d, Supplementary Table 1; the amino acid numbers are based on the human protein). The vast majority of de novo POGZ mutations identified in patients with NDDs are nonsense and frameshift mutations and distributed between the C2H2 Zn finger and centromere protein-B-like DNA-binding (CENP-DB) domains and in the CENP-DB domain itself (Fig. 1a, c). In contrast, all de novo POGZ mutations identified in unaffected controls (control de novo mutations) are missense mutations (Fig. 1b, c, Supplementary Table 1). Interestingly, while control de novo missense mutations are uniformly distributed in POGZ, many NDDs-related nonsense and frameshift mutations are positioned just upstream of the CENP-DB domain, implying that impaired CENP-DB domain function could contribute to the risk of NDDs, including ASD (Fig. 1d). We examined the deleterious effect of sporadic-ASD-associated de novo missense mutations within the CENP-DB domain and nonsense mutations resulting in the elimination or truncation of the CENP-DB domain. Since the amino acid sequences of the human and mouse POGZ are very similar (93.9% identified in amino acid sequence) (Supplementary Fig. 1), we think that each mouse mutation is likely to correspond to the respective human mutation. Previous studies have suggested that POGZ is localized to the nucleus and functions as a chromatin regulator, we therefore assume that the ASD-related de novo mutations may alter the nuclear localization of POGZ. To examine this possibility, we firstly conducted immuno-cytochemical experiments using ASD-related missense mutants, E1036K (E1040K in human POGZ)- and Q1038R (Q1042R in human POGZ)-mutated POGZ, as well as E1043X (E1047X in human POGZ)-mutated POGZ, the longest nonsense-mutated POGZ, and found that these mutations partially impaired the nuclear localization of POGZ (Supplementary Fig. 2). We next performed cellular fractionation experiments and determined that, in contrast to the nuclear localization of overexpressed wild-type (WT)-mouse (m) POGZ, R997X (R1001X in human POGZ)-, R1004X (R1008X in human POGZ)-, and E1043X (E1047X in human POGZ)-mPOGZ mutants, which entirely or partially lack the CENP-DB domain exhibited aberrant distribution in the cytoplasm (Fig. 1e, f; the amino acid numbers are based on the mouse protein). We also observed that the E1036K (E1040K in human POGZ)- and Q1038R (Q1042R in human POGZ)-mPOGZ mutants also exhibited aberrant distribution in the cytoplasm (Fig. 1g, h; the amino acid numbers are based on the mouse protein). Interestingly, in contrast to the ASD-related mutants, the de novo R1005H1, F1051L-, and H1084R-mPOGZ mutants, which harbor missense mutations within or adjacent to the CENP-DB domain identified in unaffected controls (Fig. 1b, d), showed similar protein expression patterns to WT-mPOGZ (Fig. 1i, j; the amino acid numbers are based on the mouse protein). Additionally, we performed cellular fractionation experiments using human SH-SY5Y cells and the human Q1042R-mutated POGZ and obtained essentially the same results as the results with the mouse Q1038R mutation in Fig. 1g, h (Supplementary Fig. 3). These results suggest that ASD-related de novo mutations but not control de novo mutations identified in unaffected controls impair the nuclear localization of POGZ in cells.

POGZ regulates the development of mouse neural stem cells (NSCs). To elucidate the function of POGZ in the brain, we first investigated the temporal, regional, and cell type-specific expression pattern of Pogz in the mouse brain. Temporally, expression of Pogz gradually increased during embryonic neurogenesis from embryonic day 14.5 (E14.5) to E18.5 and began to decrease after birth (Fig. 2a). At E16.5, Pogz was highly expressed in the cortical NSCs and intermediate progenitor cells (IPs) in the ventricular and subventricular zones (VZ/SVZ) (Fig. 2b, c). These expression patterns suggest that POGZ plays an important role in cortical neuronal development. To determine the role of POGZ in cortical neuronal development, we knocked down the expression of Pogz using four distinct commercial shRNAs (MISSION TRC shRNA library SP1, SIGMA-Aldrich) and a miR30-based shRNA (shRNA\textsubscript{miR30}\textsuperscript{b}) targeting Pogz (Supplementary Fig. 4a, Supplementary Tables 2 and 3). Plasmids encoding each shRNA against Pogz and GFP were coelectroporated into the lateral ventricle of E14.5 mouse forebrains. The electroporated embryos were allowed to develop until E18.5 and histologically analyzed for migration of GFP\textsuperscript{+} cells in the developing somatosensory cortex. We determined that the migration of GFP\textsuperscript{+} cells was significantly inhibited by Pogz knockdown, which was roughly proportional to the knockdown efficiency of each construct (Supplementary Fig. 4b–i). The impaired migration was rescued by forced expression of WT-mPOGZ (Fig. 2d, e). Using antibodies against cortical layer markers, we then immunostained GFP\textsuperscript{+} cells whose migration was delayed by Pogz knockdown, and we determined that Pogz knockdown had little effect on the proportion of SATB2\textsuperscript{+} GFP\textsuperscript{+} (layer II/III), CTIP2\textsuperscript{+} GFP\textsuperscript{+} (layer V) and TBR1\textsuperscript{+} GFP\textsuperscript{+} (layer VI) neurons and that the GFP\textsuperscript{+} cells with delayed migration were mostly SATB2\textsuperscript{+} neurons, representing neurons with young upper cortical characteristics (Fig. 2f–k). Considering the high expression of Pogz in NSCs during cortical neuronal development (Fig. 2b, c), the delaying of migration by Pogz knockdown may be due to impaired neuronal differentiation. We analyzed the proportion of GFP\textsuperscript{+} NSCs, IPs, and neurons at E16.5 (2 days after in utero electroporation) and determined that Pogz knockdown increased the proportion of PAX6\textsuperscript{+} NSCs and decreased the proportion of TBR2\textsuperscript{+} IPs and SATB2\textsuperscript{+} young neurons without significantly affecting migration in the somatosensory cortex within 2 days (Fig. 3a–h). These data suggest that POGZ regulates cortical neuronal development by promoting neuronal differentiation.

POGZ mutations impair POGZ function in neuronal development. We investigated the effect of ASD-related de novo
mutations on embryonic cortical neuronal development using in utero electroporation gene delivery in E14.5 embryos. We determined that forced expression of the ASD-related mPOGZ mutants failed to rescue the Pogz-knockdown-mediated migration defect at E18.5 (Fig. 4a, b). In contrast to the ASD-related mPOGZ mutants, control mPOGZ mutants, including the R1005H-, F1051L- and H1084R-POGZ mutants, rescued the Pogz-knockdown-mediated migration defect to virtually the same level as WT-mPOGZ expression at E18.5 (Fig. 4c, d). Thus, the ASD-related de novo mutations, but not the control de novo mutations identified in unaffected controls, disrupt the function of POGZ in embryonic cortical neuronal development, impairing the pathogenicity of the ASD-related de novo mutations in cortical neuronal development. We then performed overexpression experiments using ASD-related de novo mutated POGZ using WT embryos. We determined that the expression of R1004X-, E1036K-, Q1038R- and E1043X-mutated POGZ impaired the migration of GFP+ cells in WT neurons (Supplementary Fig. 5), suggesting that the de novo mutations show a dominant-negative effect upon cell migration. Considering that the de novo-mutated POGZ showed reduced nuclear localization (Fig. 1), abnormally localized de novo-mutated POGZ in the cytoplasm might inhibit the function of endogenous POGZ (e.g., abnormally localized de novo-mutated POGZ may titrate the interaction partner of POGZ in the cytoplasm). Alternatively or in addition, WT and de novo-mutated POGZ might compete each other in the nucleus.

Neuronal differentiation is impaired in NSCs derived from a patient with sporadic ASD carrying a de novo Q1042R mutation of POGZ. We investigated the effect of the ASD-related de novo mutation on NSCs derived from a patient with sporadic ASD. We previously recruited Japanese sporadic autism trios and identified an ASD patient carrying the Q1042R mutation of POGZ15. We established iPSC lines using immortalized B cells from patient-derived NSCs were larger than those from control patient-derived NSCs. We determined that the neurospheres obtained from that patient and an unaffected healthy control day 2, when they were in an early stage of neuronal differentiation. Then the number of MAP2+ neurons was analyzed at day 4, when they were in an early stage of neuronal differentiation. The proportion of MAP2+ neurons was significantly lower in the patient-derived NSCs than in the control NSCs, suggesting that neuronal differentiation is impaired in the patient-derived NSCs (Fig. 4e, f). We then analyzed the self-renewal activity of the patient-derived NSCs. We determined that the neurospheres from patient-derived NSCs were larger than those from control NSCs and that the patient-derived NSCs exhibited higher...

**Fig. 2 POGZ regulates the mouse cortical neuronal development.** a Temporal expression patterns of Pogz mRNA in the brain (each n = 3). E embryonic day; wk week-old. b Regional expression pattern of Pogz mRNA in the E16.5 brain (each n = 3). VZ ventricular zone; SVZ subventricular zone. c Fluorescence in situ hybridization of coronal sections of the E16.5 brain for Pogz and immunostaining with antibodies against Pax6, Sox2, and Tbr2. Scale bars, 100 μm. LV lateral ventricle; VZ ventricular zone; SVZ subventricular zone; IZ intermediate zone. d Impairment of neuronal migration by shRNA-mediated knockdown of Pogz in E18.5 mouse cortices electroporated at E14.5. CP cortical plate; IZ intermediate zone; SVZ subventricular zone. Scale bars, 50 μm. e Quantification of GFP+ cells in each layer (each n = 4). f GFP+ neurons were co-labeled for Satb2 (a layer II/III marker). g Quantification of GFP+ Satb2+ neurons (n = 4). h GFP+ neurons were co-labeled for Ctip2 (a layer V marker). i Quantification of GFP+ Ctip2+ neurons (n = 4). j GFP+ neurons were co-labeled for Tbr1 (a layer VI marker). k Quantification of GFP+ Tbr1+ neurons (n = 4). Note that Pogz knockdown had little effect on the proportion of Satb2+ GFP+; Ctip2+ GFP+ or Tbr1+ GFP+ neurons and that the GFP+ cells with delayed migration were mostly Satb2+ neurons. h Same slice as in f, h, j Right panels, magnifications of the areas outlined with orange boxes. White scale bars, 50 μm; orange scale bars, 10 μm. a, b, c, i, k One-way ANOVA with Bonferroni post hoc tests; a F10, 22 = 48.52; b F7, 16 = 73.23; c F2, 9 = 0.393; d F2, 9 = 1.079; e F2, 9 = 0.239. e Two-way repeated-measures ANOVA with Bonferroni post hoc tests; a F4, 27 = 48.52; b F7, 16 = 73.23; c F2, 9 = 0.393; d F2, 9 = 1.079; e F2, 9 = 0.239.
bromodeoxyuridine (BrdU) incorporation than the control NSCs (Supplementary Fig. 7 and Fig. 4g). Furthermore, we performed an in vitro migration assay in which newborn neurons migrate outwards radially from neurospheres after adhesion, and we determined that the radial migration of young neurons was significantly attenuated in the patient-derived NSCs (Fig. 4h, i). Taken together, these results show that, consistent with the impaired cortical neurogenesis induced by Pogz knockdown in mice (Fig. 4a, b), the neuronal differentiation is impaired in patient-derived NSCs.
Fig. 3 POGZ regulates the neuronal differentiation of mouse cortical neural stem cells. a Slight, non-significant migration defects caused by shRNA-mediated knockdown of Pogz in E16.5 mouse cortices electroporated at E14.5. CP cortical plate; IZ intermediate zone; SVZ subventricular zone; VZ ventricular zone; E embryonic day. Scale bars, 50 μm. b Quantification of GFP+ cells in each layer (each n = 4). CP cortical plate; IZ intermediate zone; SVZ subventricular zone; VZ ventricular zone. c Increased number of PAX6+ NSCs caused by Pogz knockdown in E16.5 mouse cortices electroporated at E14.5. d Quantification of PAX6+ cells (each n = 4). e Decreased number of TBR2+ differentiated IPs caused by Pogz knockdown in E16.5 mouse cortices electroporated at E14.5. f Quantification of TBR2+ cells (each n = 4). g Decreased number of SATB2+ differentiated neurons caused by Pogz knockdown in E16.5 mouse cortices electroporated at E14.5. h Quantification of SATB2+ cells (each n = 4). i, e, g Right panels, magnifications of the areas outlined with orange rectangles. Arrowheads indicate co-labeled cells. White scale bars, 50 μm; orange scale bars, 10 μm. b Two-way repeated-measures ANOVA with Bonferroni post hoc tests, F(2, 27) = 1.861. d, f, h One-way ANOVA with Bonferroni post hoc tests; F2, 9 = 18.37; F2, 9 = 5.710; h, F2, 9 = 11.91. *P < 0.05, **P < 0.01. Data are presented as the mean ± s.e.m.

Generation of POGZWT/Q1038R mice using CRISPR-Cas9 gene editing. To reveal the functional significance of the ASD-related Q1042R mutation in brain development and behavioral characteristics, we generated POGZWT/Q1038R mice heterozygous for the Q1038R mutation, corresponding to the human Q1042R mutation, using CRISPR-Cas9 gene editing (Supplementary Fig. 8a, b). Heterozygous POGZWT/Q1038R mice were born in the ratio predicted by Mendelian genetics (Supplementary Fig. 8c), and they exhibited reduced body size and brain in adulthood compared to WT mice (Supplementary Fig. 8d–h). We next measured the thicknesses of cortical layers in the somatosensory cortex and found that the thickness of layers II–IV and V in POGZWT/Q1038R mice were slightly decreased and increased, respectively (Supplementary Fig. 8i–n). Although POGZWT/ Q1038R mice exhibited decreased brain size, we did not find any drastic histological abnormalities, such as heterotopias, in the cortex of POGZWT/Q1038R mice. We histologically examined patient-related non-neurological abnormalities in adult POGZWT/Q1038R mice and found that POGZWT/Q1038R mice did not exhibit any significant changes in peripheral organs, including...

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eye, cochlea, trachea, stomach, duodenum, ileum, cecum and colon, compared to WT mice (Supplementary Fig. 9). Additionally, we did not find diaphragmatic hernia in adult POGZWT/ Q1038R mice. Furthermore, we performed micro-CT scanning of adult POGZWT/Q1038R mice. We did not find any significant abnormalities in the skull of POGZWT/Q1038R mice (Supplementary Fig. 10).

In addition, no homozygous point mutant (POGZQ1038R/Q1038R) offspring were produced by mating male and female POGZWT/Q1038R mice (0 of 186 pups; Supplementary Fig. 11a). We performed micro-CT scanning of mouse embryos and found that POGZWT/Q1038R mouse embryos (E15.5) showed a ventricular septal defect, which likely results in embryonic lethality (n = 4) (Supplementary Fig. 11b).

Embryonic cortical neuronal development is impaired in POGZWT/Q1038R mice. We examined embryonic cortical neuronal development and determined that the density of the SATB2+ neurons (layer II/III) was decreased in the upper layer and increased in the lower layer in the developing somatosensory cortex (Supplementary Fig. 12a, b), indicating the abnormal distribution of SATB2+ cortical excitatory neurons in POGZWT/Q1038R mice at E18.5. To confirm the impairment of cortical neuronal development in POGZWT/Q1038R mice, we labeled the new-born neurons with BrdU at E14.5. We determined that POGZWT/Q1038R mice exhibited a decreased number of SATB2+ BrdU+ cells in the upper layer and an increased number of SATB2+ BrdU+ cells in the lower layer in the developing cortex (Supplementary Fig. 12c, d). These results suggest that POGZWT/Q1038R mice exhibit impaired embryonic cortical neuronal development, which is consistent with the impaired neuronal development in the NSCs derived from the ASD patient carrying the de novo Q1042R mutation in POGZ (Fig. 4e–i).

We also histologically examined the distribution of CUX1+ cortical neurons (layer II/III) and determined that CUX1+ excitatory neurons were still abnormally distributed in the adult POGZWT/Q1038R mice (Supplementary Fig. 12e, f). There were no significant changes in the average density of the CUX1+ neurons (WT, 1126 ± 36.86 cells per mm²; POGZWT/Q1038R, 1105 ± 52.64 cells per mm²). In contrast to excitatory neurons, the average density and distribution of GABA+ interneurons were indistinguishable between WT and POGZWT/Q1038R mice (Supplementary Fig. 12g, h; the average density of GABA+ interneurons, WT, 121.6 ± 3.710 cells per mm²; POGZWT/Q1038R, 120.9 ± 2.527 cells per mm²).

Transcriptional networks underlying neuronal development is altered in NSCs derived from both the ASD patient carrying the Q1042R mutation of POGZ and POGZWT/Q1038R Mice. Given that POGZ interacts with HP1 and CHD435 and is suggested to bind DNA, POGZ may modulate the neuronal differentiation of NSCs through regulation of gene expression. To examine this possibility, we performed RNA-sequencing on NSCs derived from the ASD patient carrying the de novo Q1042R mutation of POGZ and E16.5 embryonic cortex of POGZWT/ Q1038R mice (significant results are shown in Supplementary Tables 4 and 5). We then analyzed gene ontology (GO) annotation of the differentially expressed genes between the unaffected healthy control and patient, and WT and POGZWT/Q1038R mice and found that the differentially expressed genes in human and mice were commonly enriched for GO annotations involving cellular and organismal development, particularly neuronal development (Supplementary Fig. 13a). In particular, 78 out of 913 and 251 genes annotated to neurogenesis (GO: 0022008) in human and mouse, respectively, showed commonly differential expression between human and mouse (Supplementary Tables 4 and 5). Considering that POGZ represses gene transcription in hematopoietic cells37,38, we focused on the upregulated genes in NSCs derived from the patient and POGZWT/Q1038R mice. We found that, among these differentially expressed genes involving neuronal development, a Notch ligand, Jagged canonical Notch ligand 2 (JAG2), was expressed approximately two-fold higher in NSCs derived from both the patient (fold change = 1.970) and POGZWT/Q1038R mice (fold change = 2.175) compared to each corresponding control NSCs (Supplementary Tables 4 and 5). To investigate whether POGZ binds to the Jag2 promoter in NSCs, we performed chromatin immunoprecipitation (ChIP) assays using cortical NSCs derived from E16.5 WT mice. We found that chromosome containing the Jag2 promoter was enriched by anti-POGZ antibodies, suggesting that POGZ binds to the Jag2 promoter (Supplementary Fig. 13b, c). Together with the fact that Notch signaling negatively regulate neuronal differentiation of NSCs39,40, these results suggest that POGZ may facilitate neuronal development by inhibiting gene expression, including JAG2.
The activity of excitatory cortical neurons is increased in POGZWT/Q1038R mice. Previous studies have suggested that an altered cellular balance of excitation and inhibition (E/I balance) within neural circuitry may cause the social and cognitive deficits that characterize ASD41,42. We next focused on whole-brain expression in the whole brains of 10-week-old WT and POGZWT/Q1038R mice at postnatal day 4 (each n = 19). USV ultrasonic vocalization. 

**Fig. 5 NDDs-related behavioral abnormalities in POGZWT/Q1038R mice.** a Distance traveled in the open-field test (each n = 12). b Time spent sniffing in the reciprocal social interaction test (each n = 13). c Time spent contacting in the juvenile playing test (each n = 10). d Time spent grooming in the self-grooming test (each n = 10). e Numbers of ultrasonic calls made by WT and POGZWT/Q1038R mice. f Total duration of ultrasonic calls (each n = 19). g Altered ultrasonic call patterns in POGZWT/Q1038R mice (WT, n = 17; POGZWT/Q1038R, n = 18) (Cx complex; H harmonics; T two-syllable; U upward; D downward; Ch chevron; S shorts; Cp composite; Fs frequency steps; Fl flat). WT wild-type. a–g One-way ANOVA; a F1, 22 = 0.277; b F1, 22 = 5.771; c F1, 24 = 13.02; d F1, 18 = 5.914; e F1, 18 = 13.62; f F1, 18 = 10.64; g F1, 18 = 5.465. h Two-way repeated-measures ANOVA with Bonferroni-Dunn post hoc tests, F13, 330 = 3.376. *P < 0.05, **P < 0.01. Data are presented as the mean ± s.e.m.

had an increased density of dendritic spines in the pyramidal neurons in layer II/III of the ACC (Supplementary Fig. 15e, f). We then evaluated excitatory neurotransmission in 10-week-old POGZWT/Q1038R mice using whole-cell patch-clamp recordings from pyramidal neurons in layer II/III of the ACC. Whereas there were no changes in amplitude of miniature excitatory post-synaptic currents (mEPSCs) between WT and POGZWT/Q1038R neurons, the frequency of mEPSCs was drastically increased in POGZWT/Q1038R neurons compared to WT neurons (Supplementary Fig. 15g–k). Despite the decrease in the number of the excitatory pyramidal neurons in the upper cortical layer (Supplementary Fig. 12e, f), these results suggest that the excitatory neurons in the cerebral cortex is hyperactivated during the social interaction task in POGZWT/Q1038R mice.

Treatment with an anti-epileptic agent, perampanel, improves the social deficits in POGZWT/Q1038R mice. Given that POGZWT/Q1038R mice showed the elevated activation of excitatory neurons after social interaction and abnormally activated excitatory synaptic transmission, we investigated whether pharmacological inhibition of AMPA-mediated synaptic transmission could rescue the impaired social interaction typical of POGZWT/Q1038R mice. According to the previous studies, we
determined the minimum doses of NBQX and perampanel for antiepileptic activity. With these doses, we found that 10 mg/kg of NBQX did not affect the locomotor activity and that 3 mg/kg of perampanel tended to slightly decrease the locomotor activity in the open field, which is not statistically significant (Supplementary Fig. 16a, c). We intraperitoneally administered 10 mg/kg of NBQX, a competitive AMPA receptor antagonist, to POGZWT/Q1038R mice 30 min prior to the reciprocal social interaction test and determined that NBQX treatment effectively rescued the time spent that POGZWT/Q1038R mice spent sniffing intruder mice without affecting sniffing time in WT mice (Supplementary Fig. 16b). We also administered perampanel, a negative allosteric modulator of the AMPA receptor approved by the European Medicines Agency (EMA), the US Food and Drug Administration (FDA), and Japanese Pharmaceuticals and Medical Devices Agency (PMDA) for epilepsy treatment. Interestingly, oral administration of 3 mg/kg of perampanel successfully rescued sniffing time in POGZWT/Q1038R mice (Supplementary Fig. 16d). These data suggest that the impaired social interaction observed in POGZWT/Q1038R mice is likely to be caused by hyperactivation of excitatory synaptic transmission.

Discussion

Analyzing functional mutations in individual putative causative genes for ASD is important for gaining mechanistic and pharmacological insights into ASD. Recent genetic and epidemiological studies suggest that the compromising of POGZ function by de novo mutations is likely to be involved in ASD; however, the contribution of de novo POGZ mutations to ASD onset remains largely unclear. In silico prediction shows that 17 out of 19 missense mutations identified in unaffected controls as well as 6 out of 7 NDD-related missense mutations are suggested to be damaging by at least one out of four predictive tools, namely, PROVEAN, SIFT, PolyPhen2, MutationTaster, CADD score, and The American College of Medical Genetics and Genomics (ACMG) classification (Table 1); in silico prediction of the pathogenicity of missense mutations is thus challenging and biologically assessing the pathogenicity of de novo mutations is important for understanding the etiology of ASD. Here, we assessed the pathogenicity of de novo POGZ missense mutations in vitro and in vivo and showed that the ASD-related de novo mutants, but not the control de novo mutants identified in unaffected controls, disrupt the nuclear localization of POGZ and embryonic cortical development. We also developed a new mouse model carrying a de novo POGZ mutation identified in an ASD patient. In addition to the model mouse, we established iPSC lines from the ASD patient with de novo-mutated POGZ. By comprehensively examining these human and mouse materials, we provide the first in vivo evidence suggesting that ASD-associated de novo mutations in a high-confidence ASD gene cause a wide range of aspects of the ASD phenotype.

In this study, using knockdown approaches, we provided the first in vivo evidence suggesting that POGZ regulates cortical excitatory neuron development by promoting neuronal differentiation (Figs. 2 and 3). Importantly, we determined that the neuronal developmental gene expression, including jag2, is suggested to be directly regulated by POGZ (Supplementary Fig. 13). Considering that POGZ is suggested to form part of a nuclear complex with CHD4 as well as SPI1 and Fhip1, it is likely to regulate transcriptional networks controlling neuronal differentiation through chromatin remodeling.

We determined that the ASD-associated de novo mutations of POGZ decreased the nuclear localization of the POGZ protein, impairing its function and compromising cortical excitatory neuron development (Figs. 1e–h and 4a, b). Post-mortem studies have found that developmental abnormalities associated with neuronal migration can occur in ASD; several ASD-associated gene products, such as CHD8, RELN, CNTNAP2, AUTS2, WDFY3, and TBR1, are differentially involved in excitatory neuron development. Further studies will be important for elucidating the molecular link between altered excitatory neuron development and ASD phenotypes.

POGZWT/Q1038R mice sufficiently recapitulated the pathogenic abnormalities in patients with NDDs (Supplementary Figs. 5, Supplementary Figs. 8, 12). Developmentally, consistent with the observation that 13 out of 34 patients with NDDs who carry de novo POGZ mutations are diagnosed with microcephaly, we found that 10 mg/kg of perampanel, an anti-epileptic negative allosteric modulator of the AMPA receptor and determined that the administration of perampanel also rescued the social deficits in POGZWT/Q1038R mice (Supplementary Fig. 16b). These data suggest that the impaired social interaction observed in POGZWT/Q1038R mice is likely to be caused by hyperactivation of excitatory synaptic transmission.

Methods

Ethics statement. This study was carried out in accordance with the World Medical Association’s Declaration of Helsinki and was approved by the Research Ethics Committee in Osaka University (#28-8-1). All recombinant DNA experiments were reviewed and approved by the Gene Modification Experiments Safety Committee at Osaka University (#04389). The animal experiments were performed in accordance with the guidelines for animal use issued by the Committee of Animal Experiments, Osaka University, Jikei University School of Medicine and RIKEN Tsukuba Branch, and were approved by the Committee in Osaka.
### Table 1

| Amino-acid change | PROVEAN | SIFT | PolyPhen2 | MutationTaster | CADD score | ACMG classification | Case                  |
|-------------------|---------|------|-----------|----------------|------------|---------------------|-----------------------|
| S314N             | 7.01    | 0.000 | 0.999     | 0.997          | 26.60      | Likely pathogenic ASD |
| Y597C             | 7.66    | 0.001 | 0.989     | 1.000          | 25.30      | Likely pathogenic ASD |
| H641Q             | 1.81    | 0.014 | 0.985     | 0.999          | 26.10      | Likely pathogenic ASD |
| F1051L            | 0.62    | 0.452 | 0.573     | 0.998          | 18.31      | Likely benign        |
| N136S             | 0.47    | 0.054 | 0.384     | 0.923          | 16.72      | Uncertain significance |
| N159D             | 0.09    | 0.099 | 0.999     | 0.999          | 16.72      | Uncertain significance |
| N341S             | 0.45    | 0.013 | 0.261     | 0.967          | 23.10      | Uncertain significance |
| R374Q             | 0.09    | 0.099 | 0.999     | 0.999          | 23.10      | Uncertain significance |
| R379Q             | 0.09    | 0.099 | 0.999     | 0.999          | 23.10      | Uncertain significance |
| P446L             | 6.15    | 0.003 | 0.994     | 0.999          | 24.20      | Uncertain significance |
| R674C             | 2.53    | 0.03  | 1.000     | 1.000          | 32.00      | Uncertain significance |
| D828N             | 3.68    | 0.247 | 0.835     | 1.000          | 23.80      | Uncertain significance |
| F1051L            | 0.62    | 0.452 | 0.573     | 0.998          | 18.31      | Likely benign        |
| R674C             | 2.53    | 0.03  | 1.000     | 1.000          | 32.00      | Uncertain significance |

**Antibodies**

The primary antibodies used for immunoblotting were rabbit anti-POGZ (SIGMA-Aldrich, #AV39172, 1:1000), mouse anti-Myc (9E10) (Santa Cruz Biotechnology, CA, USA, sc-40, 1:400), rabbit anti-Lamin A/C (Cell Signaling Technology, MD, USA, #2032, 1:1000), and mouse anti-a-Tubulin (DM1A) (SIGMA-Aldrich, #T9026, 1:5000); the secondary antibodies used for immunoblotting were horseradish peroxidase (HRP)-conjugated goat anti-rabbit IgG (Santa Cruz Biotechnology, #sc-2004, 1:1000), HRP-conjugated goat anti-mouse IgG (Santa Cruz Biotechnology, #sc-2005, 1:1000), alkaline phosphatase (AP)-conjugated goat anti-rabbit IgG (Santa Cruz Biotechnology, #sc-2007, 1:1000), and AP-conjugated goat anti-mouse IgG (Santa Cruz Biotechnology, #sc-2008, 1:1000). The primary and secondary antibodies used for immunostaining were rabbit anti-POGZ (Bethyl Laboratories, A302-510A) and normal rabbit IgG (Merck Life Sciences, Basel, Switzerland, #1120773910, 1:500). The secondary antibodies used for immunostaining were biotinylated goat anti-rabbit IgG (Vector Labs, CA, USA, #BA-1000, 1:200), biotinylated goat anti-mouse IgG (Vector Labs, #BA-9200, 1:200), Alexa Fluor 488-conjugated goat anti-rabbit IgG (Life Technologies, CA, USA, #A-11008, 1:200), Alexa Fluor 488-conjugated goat anti-chicken IgY (Life Technologies, #A-11039, 1:500), Alexa Fluor 647-conjugated goat anti-rat IgG (Life Technologies, #A-21217, 1:200), and Alexa Fluor 594-conjugated donkey anti-mouse IgG (Jackson Immunoresearch, PA, USA, #715-585-150, 1:250). The primary antibodies used for immunocytochemistry of ASd patient-derived and control iPSC lines were mouse anti-TRA-1-60 (1:1000), mouse anti-TRA-1-81 (1:500), rabbit anti-SOX2 (1:800), and rabbit anti-OCT-4A (1:800), which were included in the Stem Light Pluripotency Antibody Kit (Peprotech, IL, USA, 1:50). The antibodies used for ChIP were rabbit anti-POGZ (Bethyl Laboratories, TX, USA, A302-509A), rabbit anti-PGZ (Bethyl Laboratories, A302-510A) and normal rabbit IgG (Merck Millipore, 12-370).

**Neuro2a cell culture and transfection**

Mouse neuroblastoma Neuro2a cells (ATCC CCL-131) were cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with high glucose, GlutaMAX (Life Technologies), and 10% fetal bovine serum. Neuro2a cells were transfected using GeneJet In Vitro Transfection Reagent for Neuro-2A Cells (Ver. II) (SignaGen Laboratories, MD, USA). The cells were fixed with 4% PFA in PBS for 10 min at room temperature or harvested and lysed with radio-immunoprecipitation assay buffer or a Cytoplasmic & Nuclear Protein Extraction Kit (101Bio, CA, USA) 3 days after transfection.

**SHSY-5Y cell culture and transfection**

Human neuroblastoma SHSY-5Y cells (ATCC CRL-2266) were cultured in DMEM with low glucose (Nissui, Tokyo, Japan) supplemented with 4 mM L-glutamine and 10% fetal bovine serum. SHSY-5Y cells were transfected using Lipofectamine 2000 (Invitrogen, CA, USA), Lysates were resolved on 6% SDSPAGE and transferred to polyvinylidene difluoride membranes. Subsequently, these membranes were probed with the indicated primary antibodies overnight at 4°C, followed by incubation with the indicated secondary antibodies for 1 h at room temperature. Proteins were visualized by AP reaction using CDP star (Roche Life Sciences) and HRP reaction using Western Lightning Plus ECL.
Assay for nuclear localization of mutant POGZ. Cytosolic and nuclear fractions from Neuro2a cells expressing Myc-tagged WT or mutant POGZ were prepared using a CytoSpin & Nuclear Protein Extraction Kit (101Bio) according to the manufacturer's protocol. Those fractions were subjected to immunoblotting with antibodies against Myc (Lamin A/C (a nuclear marker), and α-Tubulin (a cytosolic marker)). The nuclear localization of POGZ was calculated as the ratio of the band intensity of Myc-POGZ in the nuclear fraction to that of total Myc-POGZ in the cytosolic and nuclear fractions combined.

Reverse transcription and real-time PCR. Total RNAs from cultured cells and tissues were isolated using the PureLink RNA Micro Kit (Thermo Fisher Scientific) and Purification Kit (Thermo Fisher Scientific) according to the manufacturer's instructions. The total RNAs were reverse transcribed with Superscript III (Life Technologies). Real-time PCR was performed with SYBR Premix Ex Taq (Takara Bio Inc., Shiga, Japan) using CFX96 real-time PCR detection system (Bio-Rad Laboratories, CA, USA) as described previously12. The expression levels of Pog (forward primer sequence: 5′-CCCTAATCTTGGCAGTATTTCT-3′; reverse primer sequence: 5′-CTCTGGAACATGACTTTGTTG-3′) were normalized to those of Gapdh and were determined according to the 2−ΔΔCt method.

Immunohistochemistry. E16.5, E17.5, and E18.5 mouse brains were fixed with 4% paraformaldehyde (PFA) in PBS overnight at 4 °C. The brains were sectioned at a 20 μm thickness by using a cryostat (Leica, Wetzlar, Germany, CM1520). Brains from 10-week-old adult male mice were perfused with 4% PFA in PBS and post-fixed with 4% PFA in PBS at room temperature overnight. The brains were sectioned at a 20 μm thickness by using a cryostat (Leica) for CUX1 staining after antigen retrieval methods or sectioned at a 50 μm thickness with a LinearSlicer PROTN (DOSAKA EM CO., LTD, Kyoto, Japan) for GABA staining. The brain slices were permeabilized with blocking solution containing 0.25% Triton X-100 (Wako), 1% normal goat serum (Thermo Fisher Scientific), and 1% bovine serum albumin (SIGMA-Aldrich) in PBS for 1 h at room temperature, and then incubated with the blocking solution combined with primary antibodies. The following day, the slices were incubated with the blocking solution combined with biotin-dye or fluorescent-dye conjugated secondary antibodies (Jackson ImmunoResearch Laboratories, CA, USA) in PBS for 1 h at room temperature. The biotinylated secondary antibody was labeled with Texas Red-conjugated secondary antibody and Hoechst 33258 dye (Calbiochem) for 1 h at room temperature. Those fractions were subjected to immunoblotting with antibodies against Myc, Lamin A/C (a nuclear marker), and α-Tubulin (a cytosolic marker). The nuclear localization of POGZ was calculated as the ratio of the band intensity of Myc-POGZ in the nuclear fraction to that of total Myc-POGZ in the cytosolic and nuclear fractions combined.

Fluorescence in situ hybridization. For fresh samples, E16.5 mouse embryos were rapidly frozen using dry ice. The samples were cut at a thickness of 20 μm by using a cryostat (Leica) and collected on Matsuhashi adhesive silane (MAS)-coated glass slides (Matsunami Glass Ind., Ltd, Osaka, Japan). A cRNA probe sequence targeting Pog (NCBI Reference Sequence: NM_172683.3) from base 1062 to base 1563 was amplified from mPOGZ cDNA (DNAFORM; clone ID: 30745658) via PCR and subcloned into a plasmid vector pBluescript (KS+)

Generation of iPSC lines. In utero electroporation. In utero electroporation was performed on E14.5 embryos from timed-pregnant WT ICR mice (SLC, Shizuoka, Japan)27. The pregnant mice were anesthetized by intraperitoneal injections with a solution containing 0.3 mg medetomidine (Dormitor, Zenoa Nippon Zenyaku Kogyo, Fukushima, Japan), 4 mg midazolam (Dormicum, Astellas Pharma Inc., Tokyo, Japan), and 5 mg butorphanol (Bertilpar, MP AGCO Co., Ltd.; Hokkaido, Japan) per kg. After the uterine horns were exposed and the plasmid (2.5 μg/μL) mixed with Fast Green (0.1 mg/mL, SIGMA-Aldrich) were injected into the lateral ventricles. For the knockdown of Pog and overexpression of WT-POGZ or POGZ mutants, MISSION shRNA constructs or mir30-based shRNA constructs (1 μg/μL) and pcDNA3 expression constructs encoding WT-POGZ or POGZ mutants (1 μg/μL) were injected into the lateral ventricle data were collected using the T3 RNA polymerase (Roche Life Sciences). FISH for tbr2+ cells was performed using the patient’s communication skills had been noted at a check-up when she was 18 months old. She could not communicate well with others of similar age and screeched if she was forced to go to school. She had grammatical language impairment, specifically in subject–predicate relations, and had sound sensitivity as well. She persisted in asking about specific subjects or activities that interested her. P1388: The patient was a 16-year-old Japanese female. She met the criteria for PDD-Not Otherwise Specified (NOS), which refers to ASD. The patient had no physical or mental disabilities. There was no abnormality in the results of her brain MRI or blood test. The patient’s PARS and AQ scores were both above the cut-off point. Her scores on the WAIS-III were as follows: Full-scale IQ, 65; Verbal IQ, 70; Performance IQ, 65; Verbal Comprehension Index, 73; Working Memory Index, 90; Perceptual Organization Index, 65; and Processing Speed Index, 78. A delay in the patient’s communication skills had been noted at a check-up when she was 18 months old. She could not communicate well with others of similar age and screeched if she was forced to go to school. She had grammatical language impairment, specifically in subject–predicate relations, and had sound sensitivity as well. She persisted in asking about specific subjects or activities that interested her. P1399: The subject was a 49-year-old Japanese male, the father of the patient. Generation of iPSC lines. iPSC lines were generated using immobilized B cells obtained from the ASD patient carrying C104Q2 RGR mutation and from her unaffected father28. Plasmid vectors for induction of pluripotency, including 0.63 μg pCE-hOCT3-4, 0.63 μg pCE-hUSK, 0.63 μg pCE-hUL, 0.63 μg pCE-mp3SD/I, and 0.50 μg pCBX-EBNA1 (Addgene, MA, USA) were electroporated into the immortalized B cells using the Nucleofector 2D Device (Lonza, Basel, Switzerland) with the Amaxa Human T-cell Nucleofector Kit (Lonza). The immortalized B cells introduced with the reprogramming factors were cultured in Roswell Park Memorial Institute (RPMI) medium (Wako) containing 10% fetal bovine serum for 1 week. Subsequently, the electroporated immortalized B cells were cultured with immortalized T helper cells (supT cells (REPOFELL Inc., Kanagawa, Japan) and cultured for 20–30 days in DMEM/F12 (Thermo Fisher Scientific) containing 20% KnockOut Serum Replacement (Thermo Fisher
Neural induction of iPSC lines and expansion of NSCs. The neural induction of iPSC lines was performed with PSC Neural Induction Medium (Thermo Fisher Scientific) according to the manufacturer's instructions. For neural induction, iPSC lines were cultured in Essential 8 medium (Thermo Fisher Scientific) under feeder-free conditions on Matrigel (Corning, NY, USA). On day 0 of neural induction, ~24 h after the culture were split, the culture medium was replaced with PSC neural induction medium containing neurobasal medium and PSC neural induction supplement. The neural induction medium was exchanged every other day from day 0 to day 4 of neural induction and every day after day 4 of neural induction. On day 7 of neural induction, NSCs (P0) were harvested and expanded in neural expansion medium containing 50% neurobasal medium (Thermo Fisher Scientific), 50% advanced DMEM/F12 (Thermo Fisher Scientific), and neural induction supplement (Thermo Fisher Scientific) on Matrigel. Expanded NSCs after passage 6 were used for subsequent assays.

Neuronal differentiation assay of NSCs derived from iPSC lines. ASD-patient-derived and control NSCs were seeded in Brain Phys basal medium (Nacalai Tesque, Kyoto, Japan)-based neuronal differentiation medium containing 1% N2 supplement (Wako), 2% B27 supplement (Thermo Fisher Scientific), and 50% advanced DMEM/F12 (Thermo Fisher Scientific), and neural induction supplement (Thermo Fisher Scientific) on Matrigel. Expanded NSCs after passage 6 were used for subsequent assays.

Proliferation and migration assays of neurospheres. ASD-patient-derived and control NSC suspension in DMEM/F12 (Sigma-Aldrich) containing 15 mM HEPES buffer (Sigma-Aldrich), 2% B27 supplement (Thermo Fisher Scientific), 20 ng/mL human epidermal growth factor (EGF; PeproTech), 20 ng/mL human basic fibroblast growth factor (bFGF; PeproTech), 10 ng/mL human leukemia inhibitory factor (LIF; Merck Millipore), and 0.1% heparin sodium solution 1000 IU/5 mL (Nippon Zenyaku Kogyo, Fukushima, Japan) were placed into a 96-well V-bottom plate coated with poly-l-ornithine (Sigma-Aldrich), 6.67 μg/mL human fibronectin (Thermo Fisher Scientific), and 6.67 μg/mL mouse laminin (Thermo Fisher Scientific). On day 2, which was in an early stage of neuronal differentiation, the cells were fixed with 4% PFA for 15 min at room temperature. Immunocytochemistry was performed as described previously. Briefly, the cells were permeabilized with 0.3% Triton X-100 (Wako) in PBS for 10 min and incubated with blocking solution containing 1% normal goat serum in PBS for 1 h at room temperature. Then, the cells were incubated with the blocking solution combined with primary anti-MAP2 antibody for 1 h at room temperature. The images were acquired using a ToxInsight automated microscope (Thermo Fisher Scientific). The proportion of MAP2+ neurons was automatically analyzed using the same microscope.

Neurotactin ELISA. ASD-patient-derived and control NSCs were seeded in neural expansion medium. The following day, the culture medium was replaced with neural expansion medium containing 10% neurobasal medium containing 50% neurobasal medium (Thermo Fisher Scientific), 50% advanced DMEM/F12 (Thermo Fisher Scientific), and neural induction supplement (Thermo Fisher Scientific) on Matrigel. Expanded NSCs after passage 6 were used for subsequent assays.

Mouse cortical NSCs cultures. The suspension of cortical cells prepared from E16.5 embryonic mouse cortex in NSCs expansion medium containing DMEM/F-12 (Thermo Fisher Scientific), 1% N2 supplement (Wako), 20 ng/mL EGF (PeproTech), and 20 ng/mL bFGF (PeproTech) was plated into a six-well plate. The NSCs were expanded to form primary neurospheres in suspension culture for a week. The neurospheres were dissociated into single cells using trypsin (Thermo Fisher Scientific) and seeded in the NSCs expansion medium on Matrigel. The secondary NSCs were expanded in adhesion. When the cells reached 80% confluence 2 days after plating, the cells were harvested and used in the following experiments.

RNA sequence. RNA sequence and the following analysis of the aligned reads was performed as described previously. Total RNAs isolated from NSCs differentiated from the iPSC lines and mouse cortical secondary NSCs were sequenced using the Illumina HiSeq2000 system (BGI, Beijing, China) and the Illumina HiSeq2500 system (Genome Information Research Center, Osaka University, Osaka, Japan), respectively. The expression levels of each gene were analyzed based on Fragments Per Kilobase of exon per Million mapped (FPKM).

GO and pathway analysis. The TopGene Suite (https://topgene.cchmc.org/) was used for GO annotation-based functional classification of differentially expressed genes. The genes with [fold change] ≥ 1.2 were included for the analysis of Biological process (gene in annotation: 1000 ≤ n ≤ 500,000). The GO annotations were cut off at P < 0.05 and FDR < 0.05.

ChIP assay. Chromatin was isolated from secondary NSCs derived from E16.5 cortex of WT mice. ChIP was performed using ChIP-IT Express Enzymatic Magnetic ChIP Kit & Enzymatic Shearing Kit (Active Motif, CA, USA) according to the manufacturer's instructions. Briefly, NSCs at 80% confluence on 15 cm dish were crosslinked in DMEM/F12 containing 1% formaldehyde. Following nuclear extraction, chromatin was sheared by enzyme for 30 min at 37 °C. The sheared DNA was immunoprecipitated with the antibody in a solution containing protein-G magnetic beads overnight at 4 °C. The immunoprecipitated chromatin was eluted from the beads and the crosslinking was reversed. Following ChIP, the ChIP DNA was purified using a DNA purification kit (i嫌le, USA). The DNA was diluted to 1 μg/200 μL with Tris-EDTA buffer (50 mM Tris-Cl, 2 mM EDTA, pH 8.0). The DNA was incubated with 0.5 μg of protein-specific antibody at 4 °C overnight and then the immunocomplexes were captured with Protein G Agarose beads (Pierce). The DNA was eluted with 200 μL of elution buffer (200 mM NaCl, 150 mM Tris-HCl, pH 8.0, 1% SDS) for 4 h at 65 °C. After elution, the DNA was subjected to Quantitative PCR (qPCR) for enrichment of the target region.
DNA samples were purified using Chromatim IP DNA Purification Kit (Active Motif) and amplified via PCR using GenoMatrix Whole Genome Amplification Kit (Active Motif). Quantified PCR (qPCR) was performed using the amplified DNA with the primers for the mouse jag2 promoter region (forward primer 1 sequence: 5′-GGATGCCGTATTTTGTTTGT-3′; reverse primer 1 sequence: 5′-TTGGAGGAGGGCTTCTC-3′; forward primer 2 sequence: 5′-AGATGGAGAGACCCCT CCT-3′; reverse primer 2 sequence: 5′-GCACTGGGTTGTTAACA-3′; forward primer 3 sequence: 5′-GTCAGGCTGATCTGCTT-3′; reverse primer 3 sequence: 5′-TCCAGGCCCTTATACCACA-3′).67 The levels of enrichment of each amplicon by ChIP were normalized to the amount of amplified DNA fragments immunoprecipitated with each antibody.

Behavioral tests: All olfactory trials were carried out on male C57BL/6Ncl mice at 1.5–4 months of age, except the ultrasonic vocalization test and the juvenile play test. All behavioral experiments were performed during the light period by experimenters who were blind to the genotypes and treatments of the mice.

Home cage activity: Each mouse was placed in a test cage under a 12:12 h light–dark cycle (light on at 8.00). After 1 day of habituation, spontaneous activity in the light and dark phases, total activity and the ratio of activity in the light phase to total activity were measured for 5 days using an infrared activity sensor (O’Hara & Co., Ltd., Tokyo, Japan).88

Fear conditioning test: The fear-conditioning test was performed using an Image EZ4 (O’Hara & Co., Ltd.), automated fear contextual and tone-dependent fear conditioning system. On day 1, each mouse was placed into a shock chamber (Box A: 10 × 10 × 10 cm, white polystyrene boards, stainless steel rod floor, O’Hara & Co., Ltd.) for 120 s, immediately followed by the presentation of four tone–shock pairs at 90 s intervals. Each tone–shock pair included a tone (70 dB, 10 kHz) for 30 s and a 0.5 mA foot shock lasting for the final 0.1 s of the 30 s period. In contextual fear conditioning, each mouse was placed back in box A for 6 min of measurement. On day 3, each mouse was placed in a white transparent chamber (Box B) for 120 s, and then the tone was presented four times at 90 s intervals. Freezing during the first 120 s was measured as pre-tone freezing, and freezing during the tone presentations was measured as cued freezing.

Novel object recognition test: Prior to each session, mice were acclimated to the test room for at least 30 min. After 10 min of habituation to the experimental box under dim lighting conditions (10–20lx) for 3 consecutive days, the test mouse was allowed to freely explore two novel objects (A and B) in the box for 10 min. Twenty-four hours after the training session, the retention session was conducted. In the retention session, object B was replaced with novel object C, and the mouse was allowed to move freely for 10 min in the same box. The exploration time for each object was measured. The discrimination index (%) was the difference between the exploration time for the novel object and that for the familiar object divided by total exploration time. This index was used to calculate values for recognition memory. This test was conducted between 10:00 and 14:00.

Open field test: Locomotor activity was measured using the open field. Each mouse was placed in the center of the open-field apparatus (45 × 45 × 30 cm). The total distance traveled and time spent in the center area (25 × 25 cm) were recorded. Data were recorded for 90 min per mouse.

Light/dark transition test: The light/dark transition test was performed using an apparatus that consisted of two sections of equal size (20 × 20 × 25 cm, O’Hara & Co., Ltd.).68 The illumination was 353 lx in the light chamber and 0.1 lx in the dark chamber. Each mouse was placed in the middle of the light chamber and allowed to move freely. The test chamber was continuously superfused at a rate of 1.5 mL/min. The light chamber and the number of transitions between chambers were automatically measured using Image LD4 (O’Hara & Co., Ltd.) for 10 min.

Y-maze test: Each mouse was placed on the center of an apparatus consisting of three arms (arm length: 40 cm, arm bottom width: 3 cm, arm upper width: 10 cm, height of wall: 12 cm, O’Hara & Co., Ltd.).68 The distance traveled and the alteration ratio were measured.

PPI test: Each mouse was habituated to a sound-proof box (33 × 43 × 33 cm, O’Hara & Co., Ltd.) with 65 dB background noise for 5 min. In order to acclimatize mice to the startle pulse, 110 dB per 40 ms of white noise was presented during the 5 min habituation period. The startle response was measured using an UltraSoundGateCM16/CMPA microphone (Avisio Bioacoustics, Glenelg, Germany) in a sound attenuation chamber under light control (90 lx), and recorded with Avisio-SASLab Pro software (Avisio Bioacoustics). The mouse was placed in the middle of the sound attenuation chamber, and the startle pulse was presented at a rate of 120 s, immediately followed by the presentation of four tone–shock pairs at 90 s intervals. Each tone–shock pair included a tone (70 dB, 10 kHz) for 30 s and a 0.5 mA foot shock lasting for the final 0.1 s of the 30 s period. In contextual fear conditioning, each mouse was placed back in box A for 6 min of measurement. On day 3, each mouse was placed in a white transparent chamber (Box B) for 120 s, and then the tone was presented four times at 90 s intervals. Freezing during the first 120 s was measured as pre-tone freezing, and freezing during the tone presentations was measured as cued freezing.

Electrophysiology. WT and POGZ−/− mice (10–11 week-old, male) were decapitated under isoflurane anesthesia (5% in 100% O2) and the brains were quickly removed and fixed on a freezing microtome (VT1200S, Leica) with oblique illumination. Patch-clamp electrodes (4–6 MΩ) and a solution containing 30 NaHCO3, 25 D-glucose, 5 L-ascorbic acid and N-acetyl-L-cysteine were equilibrated with 95% O2 + 5% CO2 (pH 7.4) and incubated in the cutting solution at 34 °C for 15–20 min. The slices were then kept at room temperature (20–25 °C) in the standard artificial cerebrospinal fluid (ACSF) solution of (in mM) 125 NaCl, 3 KCl, 2 CaCl2, 1.25 MgCl2, 2.5 NaH2PO4, 8 glucose, 0.4 ascorbic acid, and 25 NaHCO3 (pH 7.4 bubbled with 95% O2 + 5% CO2 osmolarity, −310 mOsm/kg) until the electrophysiological recording. Each slice was transferred to a recording chamber (0.4 mL volume), and fixed with nylon grids attached to a platinum frame. The slice was submerged and continuously superfused at a rate of 1.5–2.5 mL/min with the standard ACSF at 30–32 °C. Whole cell membrane current was recorded from the pyramidal neurons in the layer II/III of the ACC visually identified under an upright microscope (BX51WI, Olympus) with oblique illumination. Patch-clamp electrodes (4–6 MΩ) were made from borosilicate glass pipettes (1B150F-4, World Precision Instruments) and filled with internal solution containing (in mM) 12.5 potassium gluconate, 10 Hepes, 17.5 KCl, 0.2 EGTA, 8 NaCl, 2 MgATP, 0.3 NaGTP (pH, 7.2: osmolarity, 290–300 mOsm).

Electrophysiology. WT and POGZ−/− mice (10–11 week-old, male) were decapitated under isoflurane anesthesia (5% in 100% O2) and the brains were quickly removed and fixed on a freezing microtome (VT1200S, Leica) with oblique illumination. Patch-clamp electrodes (4–6 MΩ) and a solution containing 30 NaHCO3, 25 D-glucose, 5 L-ascorbic acid and N-acetyl-L-cysteine were equilibrated with 95% O2 + 5% CO2 (pH 7.4) and incubated in the cutting solution at 34 °C for 15–20 min. The slices were then kept at room temperature (20–25 °C) in the standard artificial cerebrospinal fluid (ACSF) solution of (in mM) 125 NaCl, 3 KCl, 2 CaCl2, 1.25 MgCl2, 2.5 NaH2PO4, 8 glucose, 0.4 ascorbic acid, and 25 NaHCO3 (pH 7.4 bubbled with 95% O2 + 5% CO2 osmolarity, −310 mOsm/kg) until the electrophysiological recording. Each slice was transferred to a recording chamber (0.4 mL volume), and fixed with nylon grids attached to a platinum frame. The slice was submerged and continuously superfused at a rate of 1.5–2.5 mL/min with the standard ACSF at 30–32 °C. Whole cell membrane current was recorded from the pyramidal neurons in the layer II/III of the ACC visually identified under an upright microscope (BX51WI, Olympus) with oblique illumination. Patch-clamp electrodes (4–6 MΩ) were made from borosilicate glass pipettes (1B150F-4, World Precision Instruments) and filled with internal solution containing (in mM) 12.5 potassium gluconate, 10 Hepes, 17.5 KCl, 0.2 EGTA, 8 NaCl, 2 MgATP, 0.3 NaGTP (pH, 7.2: osmolarity, 290–300 mOsm).

The membrane potential was held at −60 mV. The membrane current was recorded using the MultiClamp 700B amplifier (Molecular Devices), filtered at 2 kHz and digitized at 10 kHz with a 16-bit resolution using a PowerLab interface (AD Instruments). mEPSCs were recorded in the presence of picrotoxin (100 μM) and tetrodotoxin (1 μM) and analyzed by Igor Pro 7 (WaveMetrics). 100 events in each neuron were quantified. All experiments were performed in a manner blinded to the mouse genotype during the experiments and analyses.

Drug administration. NBQX diosidum salt hydrate (Abcam) dissolved in saline was intraperitoneally administered to 10-week-old male mice at a dosage of 10 mg/kg 30 min before the open field test or reciprocal social interaction test. Perampanel was administered i.p. 30 min before the test.
powder (Toronto Research Chemicals, ON, Canada) was suspended in a 0.5% weight-per-volume methyl cellulose (400 cP, Wako) solution. Periparamellar was administered via oral gavage to 10-week-old male mice at a dose of 3 mg/kg in a volume of 20 μL/g 30 min before the open field test or reciprocal social interaction test.

Statistical analysis. The quantified data from the western blots and qRT-PCR were statistically analyzed using one-way ANOVA followed by Bonferroni post hoc tests. The quantified data from the immunohistochemistry and HE staining analysis were statistically analyzed using two-way ANOVA with repeated measures followed by Bonferroni–Dunn post hoc tests and Student’s t-test. The quantified data from in utero electroporation were statistically analyzed using one-way ANOVA and two-way ANOVA with repeated measures followed by Bonferroni–Dunn post hoc tests. The quantified data from the neuronal differentiation assay, the BrdU ELISA on NGF and the proliferation and migration assays on neurospheres were statistically analyzed using Student’s t-test. The body weights of WT and POGZ WT/Q1038R mice were statistically analyzed using two-way ANOVA with repeated measures followed by Bonferroni–Dunn post hoc tests. The micro-CT data were statistically analyzed using Welch’s t-test. The behavioral data were statistically analyzed using one-way ANOVA and two-way ANOVA followed by Bonferroni–Dunn post hoc tests. The Golgi staining data were statistically analyzed using Student’s t-test. The electrophysiological data were statistically analyzed using Mann–Whitney U test and Kolmogorov–Smirnov test for details, see the description in the figure legend. The significance level was set at P < 0.05. Statistical analyses were conducted using StatView (SAS Institute, NC, USA) and R (version 3.4.1).

In silico prediction of the effect of missense mutations. The pathogenicity of NDD-related and control de novo missense mutations and their effect on the POGZ function were predicted using PROVEAN, SIFT (http://provean.jcvi.org/), PolymiD (http://genetics.bwh.harvard.edu/pph2/), MutationTaster (http://www.mutationtaster.org/), CADD score (https://cadd.gs.washington.edu/snv), and The American College of Medical Genetics and Genomics (ACMG) classification.

Reporting summary. Further information on research design is available in the Nature Research Reporting Summary linked to this article.

Data availability. RNA sequencing data have been deposited to the DDBJ Sequence Read Archive (DRA) and are accession number DRA009486. The source data underlying Fig. 1e, g and i and Supplementary Figs. 3a, 4b, 4f, 7b, and 8d are provided as a Source Data file. All data supporting the finding of this study are available with the Article and its Supplementary Information or from the corresponding author upon the reasonable request.

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References
1. de la Torre-Ubieta, L., Won, H., Stein, J. L. & Geschwind, D. H. Advancing the understanding of autism disease mechanisms through genetics. Nat. Med. 22, 345–361 (2016).
2. Courchesne, E. et al. The ASD Living Biology: from cell proliferation to clinical phenotype. Mol. Psychiatry 24, 88–107 (2018).
3. Xu, G., Strathmann, L., Liu, B. & Bao, W. Prevalence of autism spectrum disorder among US children and adolescents, 2014–2016. JAMA 319, 81–82 (2018).
4. Christensen, D. L. et al. Prevalence and characteristics of autism spectrum disorder among children aged 8 years—Autism and Developmental Disabilities Monitoring Network, 11 Sites, United States, 2012. Morbidity Mortal. Weekly Rep. Surv. Summ. (Wash., DC: 2002) 65, 1–23 (2018).
5. Tick, B., Bolton, P., Happe, F., Rutter, M. & Rijsdijk, F. Heritability of autism spectrum disorders: a meta-analysis of twin studies. J. Child Psychol. Psychiatry 57, 585–595 (2016).
6. Gaugler, T. et al. Most genetic risk for autism resides with common variation. Nat. Genet. 46, 881–885 (2014).
7. Abrahams, B. S. et al. SFARI Gene 2.0: a community-driven knowledgebase for the autism spectrum disorders (ASDs). Mol. Autism 4, 36 (2013).
8. Carter, M. T. & Scherer, S. W. Autism spectrum disorder in the genetics clinic: a review. Clin. Genet. 83, 399–407 (2013).
9. Tamminen, K. et al. Molecular diagnostic yield of chromosomal microarray analysis and whole-exome sequencing in children with autism spectrum disorder. JAMA 314, 895–903 (2015).
10. Turner, T. N. et al. Genomic patterns of de novo mutation in simplex autism. Cell 171, 710–722 (2017).
11. Janeczko, M. et al. Advanced paternal age effects in neurodevelopmental disorders—review of potential underlying mechanisms. Transl. Psychiatry 7, e1019 (2017).
12. Deciphering Developmental Disorder Study. Prevalence and architecture of de novo mutations in developmental disorders. Nature 542, 433–438 (2017).
13. Aizenbud, L. B., Sulovari, A., Turner, T. N., Neale, B. M., P. & Eichler, E. E. Recurrent de novo mutations in neurodevelopmental disorders: properties and clinical implications. Genome Med. 9, 101 (2017).
14. De Rubeis, S. et al. Sympathetic, transcriptional and chromatin genes disrupted in autism. Nature 515, 209–215 (2014).
15. Hashimoto, R. et al. Whole-exome sequencing and neurite outgrowth analysis in autism spectrum disorder. Hum. Genet. 136, 199–206 (2016).
16. Lissiou, I. et al. The contribution of de novo coding mutations to autism spectrum disorder. Nature 515, 216–221 (2014).
17. Takata, A. et al. Integrative analyses of de novo mutations provide deeper biological insights into autism spectrum disorder. Cell Rep. 22, 734–747 (2018).
18. Li, J. et al. A comparative study of the genetic components of three subcategories of autism spectrum disorder. Mol. Psychiatry 24, 1720–1731 (2019).
19. Krumm, N. et al. Excess of rare, inherited truncating mutations in autism. Nat. Genet. 47, 582–588 (2015).
20. Sanders, S. J. et al. Insights into autism spectrum disorder genomic architecture and biology from 71 risk loci. Neuron 87, 1215–1233 (2015).
21. Tempera, A. L. et al. Germline CHD8 haploinsufficiency alters brain development in mouse. Nat. Neurosci. 20, 1062–1073 (2017).
22. Platt, R. J. et al. CHD8 mutation leads to autistic-like behaviors and impaired striatal circuits. Cell Rep. 19, 335–350 (2017).
23. Durak, O. et al. CHD8 mediates cortical neurogenesis via transcriptional regulation of cell cycle and Wnt signaling. Nat. Neurosci. 19, 1477–1486 (2016).
24. Katayama, Y. et al. CHD8 haploinsufficiency results in autistic-like phenotypes in mice. Nature 537, 675–679 (2016).
25. Jung, E. M. et al. Arid1b haploinsufficiency disrupts cortical interneuron development and mouse behavior. Nat. Neurosci. 20, 1694–1707 (2017).
26. Shibutani, M. et al. Arid1b haploinsufficiency causes abnormal brain gene expression and autism-related behaviors in mice. Int. J. Mol. Sci. 18, E1872 (2017).
27. Celen, C. et al. Arid1b haploinsufficient mice reveal neuropsychiatric phenotypes and reversible causes of growth impairment. elife 6, e25730 (2017).
28. Dentici, M. L. et al. Expanding the phenotypic spectrum of truncating POGZ mutations: association with CNS malformations, skeletal abnormalities, and distinctive facial dysmorphism. Am. J. Med. Genet. A 173, 1965–1969 (2017).
29. Yuen, R. et al. Whole genome sequencing resource identifies 18 new candidate genes for autism spectrum disorder. Nat. Neurosci. 20, 602–611 (2017).
30. White, J. et al. POGZ truncating alleles cause syndromic intellectual disability. Genome Med. 8, 3 (2016).
31. Stessman, H. A. F. et al. Disruption of POGZ is associated with intellectual disability and autism spectrum disorders. Am. J. Hum. Genet. 98, 541–552 (2016).
32. Tan, B. et al. A novel de novo POGZ mutation in a patient with intellectual disability. J. Hum. Genet. 61, 357–359 (2016).
33. Fukai, R. et al. A case of autism spectrum disorder arising from a de novo missense mutation in POGZ. J. Hum. Genet. 60, 277–279 (2015).
34. Ye, Y. et al. De novo POGZ mutations are associated with neurodevelopmental disorders and microcephaly. Cold Spring Harb. Mol. Case Stud. 1, a004555 (2015).
35. Ostapczuk, V. et al. Activity-dependent neuroprotective protein recruits HP1 and CHD4 to control lineage-specifying genes. Nature 557, 739–743 (2018).
36. Gunther, M., Laithier, M. & Brison, O. A set of proteins interacting with transcription factor Sp1 identified in a two-hybrid screening. Mol. Cell. Biochem. 210, 131–142 (2000).
37. Gudmundsdottir, B. et al. POGZ is required for silencing mouse embryonic homeostasis in autism spectrum disorders. Cell 148, 1477–1488 (2016).
38. Gunn, M., Reitman, S. & Vellai, T. Vascular/hibernatory balance and circulatory homeostasis in autism spectrum disorders. Neuron 87, 684–698 (2019).
39. Paterson, C. & Law, A. J. Toward better strategies for understanding disrupted cortical excitatory/hibernatory balance in schizophrenia. Biol. Psychiatry 83, 632–634 (2018).
40. Eguchi, M. & Yamaguchi, S. In vivo and in vitro visualization of gene expression dynamics over extensive areas of the brain. Neuroimage 44, 1274–1283 (2009).
44. Seiriki, K. et al. High-speed and scalable whole-brain imaging in rodents and primates. Neuron 94, 1085–1100 e1086 (2017).
45. Seiriki, K. et al. Whole-brain block-face serial microscopy tomography at subcellular resolution using FAST. Nat. Protoc. 14, 1509–1529 (2019).
46. Mundy, P. A review of joint attention and social-cognitive brain systems in typical development and autism spectrum disorder. Eur. J. Neuropsychol. 47, 497–514 (2018).
47. Waye, M. M. Y. & Cheng, H. Y. Genetics and epigenetics of autism: a review. Psychiatry Clin. Neurosci. 72, 228–244 (2018).
48. Gilbert, J. & Man, H. Y. Fundamental elements in autism: from neurogenesis and neurite growth to synaptic plasticity. Front. Cell. Neurosci. 11, 359 (2017).
49. Nithianantharajah, J. & Grant, S. G. Cognitive components in mice and humans: combining genetics and touchscreens for medical translation. Neuroradiol. Learn. Ment. 105, 13–19 (2013).
50. Hiroi, N. Critical reappraisal of mechanistic links of copy number variants to dimensional constructs of neuropsychiatric disorders in mouse models. Psychiatry Clin. Neurosci. 72, 301–321 (2018).
51. O’Tuathailg, C. M. P., Moran, P. M., Zhen, X. C. & Waddington, J. L. Translating advances in the molecular basis of schizophrenia into novel cognitive treatment strategies. Br. J. Pharmacol. 174, 3173–3190 (2017).
52. Sundelin, H. E. et al. Autism and epilepsy: a population-based nationwide cohort study. Neurology 87, 192–197 (2016).
53. Tuchman, R. & Rapin, I. Epilepsy in autism. Lancet Neurol. 1, 352–358 (2002).
54. Selimbeyoglu, A. et al. Modulation of prefrontal cortex excitation/inhibition balance rescues social behavior in CNTNAP2-deficient mice. Sci. Transl. Med. 9, eaax6733 (2017).
55. Yizhar, O. et al. Neocortical excitation/inhibition balance in information processing and social dysfunction. Nature 477, 171–178 (2011).
56. Nakazawa, T. et al. Emerging roles of ARHGAP33 in intracellular trafficking of TrkB and pathophysiology of neuropsychiatric disorders. Nat. Commun. 7, 10594 (2016).
57. Okamoto, M. et al. DBZ regulates cortical cell positioning and neurite development by sustaining the anterograde transport of Lsl1 and DISC1 through control of Nde1l dual-phosphorylation. J. Neurosci. 35, 2942–2958 (2015).
58. American Psychiatry Association. Diagnostic and Statistical Manual of Mental Disorders. 4th edn (DSM-IV. American Psychiatric Association, Washington, DC, 1994).
59. Yamada, A. et al. Emotional distress and its correlates among parents of children with pervasive developmental disorders. Psychiatry Clin. Neurosci. 61, 651–657 (2007).
60. Wakabayashi, A., Tojo, Y., Baron-Cohen, S. & Wheelwright, S. [The Autism-Spectrum Quotient (AQ) Japanese version: evidence from high-functioning clinical group and normal adults]. Shinrigaku kenkyu : Jpn. J. Psychol. 75, 78–84 (2004).
61. Committee]W-IP. Japanese Wechsler Intelligence Scale for Children. 3rd edn. (Nihon Bunka Kagakusha, Tokyo, 1998).
62. Nakazawa, T. et al. Differential gene expression profiles in neurons generated from lymphoblastoid B-cell line-derived iPS cells from monzygotic twin cases with treatment-resistant schizophrenia and discordant responses to clozapine. Schizophr. Res. 181, 75–82 (2017).
63. Ayabe, S., Nakashima, K. & Yoshiki, A. Off- and on-target effects of genome editing in mouse embryos. J. Reprod. Dev. 65, 1–5 (2018).
64. Yoshimi, K. et al. soDIN-mediated knock-in with CRISPR-Cas for large genomic regions in zygotes. Nat. Commun. 7, 10431 (2016).
65. Hwang, W. Y. et al. Efficient genome editing in zebrafish using a CRISPR-Cas system. Nat. Biotechnol. 31, 227–229 (2013).
66. Pietras, A., von Stedingk, K., Lindgren, D., Pahlman, S. & Axelson, H. JAG2 efficient genome editing in zebra...
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