De novo ATP1A3 variants cause polymicrogyria

Satoko Miyatake1,2,*, Mitsuhiro Kato3,*, Takuma Kumamoto4, Tomonori Hirose5, Eriko Yoshida1, Takaaki Matsui6, Hideyuki Takeuchi7, Hiroshi Doi7, Keisuke Hamada8, Mitsuku Nakashima1,9, Kazunori Sasaki5, Akio Yamashita5, Atsushi Takata1,10, Kohei Hamanaka1, Mai Satoh1, Takabumi Miyata1, Yuri Sonoda1,11, Momoko Sasazuki11,12, Hiroyuki Torisu11,12, Toshiro Hara11,13, Yasunari Sakai14, Yushi Noguchi14, Mazumi Miura14, Yoko Nishimura15, Kazuyuki Nakamura16, Hideyuki Asai3,2, Nodoka Hinokuma3, Fuyuki Miya17,18,19, Tatsuhiko Tsunoda17,18,19, Masumi Togawa20, Yukihiro Ikeda21, Nobusuke Kimura22, Kaoru Amemiya23, Asako Horino24, Masatake Fukuoka24, Hiroko Ikeda24, Goni MerHAV25, Nina Ekhilevitch26, Masaki Miura27, Takeshi Mizuguchi21, Noriko Miyake21, Atsushi Suzuki21,28, Shouichi Ogata21, Hiroto Saito21,13, Hidehisa Takahashi21, Fumiaki Tanaka21, Kazuhiro Ogata28, Chiaki Ohtaka-Maruyama4, Naomichi Matsumoto1†

Polymicrogyria is a common malformation of cortical development whose etiology remains elusive. We conducted whole-exome sequencing for 124 patients with polymicrogyria and identified de novo ATP1A3 variants in eight patients. Mutated ATP1A3 causes functional brain diseases, including alternating hemiplegia of childhood (AHC), rapid-onset dystonia parkinsonism (RDP), and cerebellar ataxia, areflexia, pes cavus, optic nerve atrophy, and sensorineural deafness (CAPOS). However, our patients showed no clinical features of AHC, RDP, or CAPOS and had a completely different phenotype: a severe form of polymicrogyria with epilepsy and developmental delay. Detected variants had different locations in ATP1A3 and different functional properties compared with AHC, RDP, or CAPOS-associated variants. In the developing cerebral cortex of mice, radial neuronal migration was impaired in neurons overexpressing the ATP1A3 variant of the most severe patients, suggesting that this variant is involved in cortical malformation pathogenesis. We propose a previously unidentified category of polymicrogyria associated with ATP1A3 abnormalities.

INTRODUCTION

Malformations of cortical development occur when neuroblast proliferation, differentiation, migration, or cortical organization is interrupted during human brain development. Polymicrogyria is a common form of malformation of cortical development and is defined by the existence of many abnormally small gyri, producing an irregular and fused cortical surface (1). Clinically, polymicrogyria causes various neurological symptoms, such as epilepsy, intellectual disability, and impaired oromotor function. Both genetic and non-genetic factors (e.g., hypoxic insults or intrauterine cytomegalovirus infection) cause this disease; however, only a minority of patients can be explained by a known genetic cause.

Na+/K+ adenosine triphosphatases (Na+/K+ ATPases) are ubiquitously expressed transmembrane ion pumps that actively export three Na+ and import two K+ using hydrolyzed adenosine 5′-triphosphate (ATP) energy. These ion pumps maintain ion gradients across the cell membrane and consist of α-, β-, and sometimes regulatory γ-subunits. The α-subunit holds cytosolic ATPase machinery and the ion-transporting membrane domain, while the β-subunit is necessary for proper trafficking to the plasma membrane and supports the functional expression and kinetic properties of the α-subunit (2). Tissue-specific isoform expressions are known among the four α- and three β-subunit isoforms that exist in humans. In the central nervous system, the α1 isoform is ubiquitously expressed, while α2 is expressed in astrocytes and α3 in neurons (3).
Dominant mutations in $ATP1A3$, which encodes the $\alpha_3$-subunit, cause characteristic functional brain diseases known as $ATP1A3$-related disorders, which have at least three distinct phenotypes: alternating hemiplegia of childhood (AHC) (4); rapid-onset dystonia parkinsonism (RDP) (5); and cerebellar ataxia, areflexia, pes cavus, optic nerve atrophy, and sensorineural deafness (CAPOS) (6). Dominant mutations in $ATP1A3$ also cause forms of developmental and epileptic encephalopathies, such as early infantile epilepsy and encephalopathy (EIEE) with or without apnea (7), relapsing encephalopathy with cerebellar ataxia (8), or fever-induced paroxysmal weak encephalopathy (9). Although AHC, RDP, and CAPOS have distinct neurological symptoms, they are recognized as part of a continuum and share some core clinical features (10).

Here, we report eight patients from unrelated families with de novo variants in $ATP1A3$, presenting with none of the features of AHC, RDP, or CAPOS. These patients had polymicrogyria with extremely severe epilepsy, intellectual disability, and sometimes progressive brain atrophy and cardiac failure. We also reveal that the specific variant localizations in $ATP1A3$ in our patients differ from those of AHC, RDP, or CAPOS in terms of three-dimensional protein structure and functional consequences. Furthermore, we show the possible association between an identified variant and defects in cortical architecture using embryonic mice expressing this variant. These results imply that our patients demonstrate a novel phenotype associated with $ATP1A3$ abnormality.

**RESULTS**

**Clinical analysis**

Clinical features are summarized in Table 1 in order of clinical severity (from patients 1 to 8). Patient 5 was Israeli, and the other patients were Japanese. No patients satisfied the diagnostic criteria for AHC, RDP, or CAPOS, but all patients had bilateral polymicrogyria of either the frontoparietal or perisylvian regions (Fig. 1 and fig. S1). Furthermore, all patients exhibited extremely severe clinical features. Of the seven patients with seizures, onset was at 1 day old in four patients and by 4 days old in three patients. Seizure types varied, including tonic-clonic, autonomic, or myoclonic seizures. Two patients had well-controlled or partially controlled seizures (patients 2 and 5), and the remaining five patients had intractable and frequent seizures. All patients had severe developmental delay, and only one achieved social smile and head control (patient 8); this patient had no seizures. At least three patients demonstrated progressive cerebral and cerebellar atrophy (patients 1, 3, and 4) (Fig. 1 and figs. S2 and S3), and one patient (patient 6) showed progressive atrophy at the cerebellum vermis only. Patient 2 underwent magnetic resonance imaging (MRI) only once, and no evaluation of brain atrophy has been performed since. Two patients (patients 1 and 2) had episodic tachycardia. This resulted in congestive heart failure in patient 1, and in patient 2, no changes in amplitude-integrated electroencephalogram were observed during the attack, suggesting that it was unlikely to be an autonomic seizure. Two patients (patients 1 and 3) had cardiac failure, which is rarely observed in typical polymicrogyria cases (see the Supplementary Materials for more clinical details).

**Genetic analysis**

Using whole-exome sequencing, we identified seven de novo variants in $ATP1A3$ (NM_152296.4) in 8 of 124 patients, a diagnostic yield of approximately 6.5%. Five variants were in-frame changes (p.Asp992del in patients 1 and 2, p.Tyr991_Ile994delinsPheAlaHisLeuHisLeu in patient 3, p.Ile994_Arg995insHisGluLeu in patient 4, p.Phe857del in patient 5, and p.Gly854_Phe856del in patient 6), and two were missense (p.Cys596Tyr in patient 7 and p.Gln895Pro in patient 8) (Table 1). All variants were confirmed by Sanger sequencing (fig. S4) and were considered pathogenic on the basis of population allele frequency and in silico predictions (table S1). Six variants were novel, while the variant in patient 5 had been previously reported in a patient with nervous system abnormality (11). The variants in patients 1, 2, and 7 were novel changes at the same amino acid residues where different substitutions had previously been reported in patients with AHC [p.Asp992Tyr (4) and p.Cys596Arg (12)]. We were unable to find further clinical information on these patients in the published reports.

**Structural considerations of the mutations identified in the Na\(^+\)/K\(^+\) ATPase $\alpha_3$-subunit**

To evaluate the impact of the identified mutations in the Na\(^+\)/K\(^+\) ATPase $\alpha_3$-subunit (hereafter called the $\alpha$-subunit), we mapped the mutation sites onto the crystal structure of $S. acanthias$ Na\(^+\)/K\(^+\) ATPase [Protein Data Bank (PDB) code: 2ZXE] (13), which Phyre2 predicts to be highly homologous to human Na\(^+\)/K\(^+\) ATPase (14). The structure of $S. acanthias$ Na\(^+\)/K\(^+\) ATPase consists of the catalytic $\alpha$- and $\beta$-subunits and regulatory $\gamma$-subunit (Fig. 2A). The $\beta$-subunit is essential for the correct integration of the $\alpha$-subunit into the lipid bilayer and for structural stabilization of the $\alpha$-subunit by preventing its degradation (15). The mutation sites in human Na\(^+\)/K\(^+\) ATPase are located in the extracellular, transmembrane, and cytoplasmic regions of the $\alpha$-subunit.

In the extracellular region, a loop (p.Asp882–Trp896) in the $\alpha$-subunit is the primary interaction site with the $\beta$-subunit (13), where the side chain of p.Gln895 ($\alpha$-subunit) forms hydrogen bonds with p.Arg182 ($\beta$-subunit) (Fig. 2B). The p.Gln895Pro mutation is therefore predicted to disrupt this interaction, possibly reducing the affinity between the $\alpha$- and $\beta$-subunits. FoldX predicted the p.Gln895Pro mutation to cause a moderate free-energy change (2.33 ± 0.39 kcal mol\(^{-1}\)) (Fig. 2F), which may affect subunit interactions.

In the transmembrane sections, the mutated regions p.Gly854–Phe857 and p.Tyr991–Arg995 of the $\alpha$-subunit interact with the $\beta$-subunit (Fig. 2A). p.Phe857 ($\alpha$-subunit) is a key residue in the interaction, mainly by making van der Waals contacts with p.Thr984 and p.Phe912, respectively, within the $\beta$-subunit (Fig. 2C). Furthermore, the $\alpha$-subunit is involved in a hydrophobic core of the $\alpha$-subunit near K\(^+\) binding sites. Thus, the identified deletion mutations of p.Gly854–Phe856 and p.Phe857 in this study are predicted to destabilize protein folding and disrupt intersubunit interactions.

In the p.Tyr991–Arg995 region of the $\alpha$-subunit, the side chain of p.Tyr991, whose conformation is maintained by a hydrogen bond with p.Gln846 and proximity to p.Ile994, makes van der Waals contacts with p.Phe38 and p.Phe42 ($\beta$-subunit) (Fig. 2D). The side chains of p.Asp992 and p.Glu993 form hydrogen bonds with the side chain of p.Arg995 and the backbone amide of p.Val934, respectively. Hence, the three identified mutations in the human $\alpha$-subunit (the insertion of a His-Glu-Ile tripeptide between p.Ile994 and p.Arg995, the insertion
Table 1. Clinical summary of the patients with ATP1A3 variants and polymicrogyria. DQ, developmental quotient; EEG, electroencephalogram; CT, computed tomography; MRI, magnetic resonance imaging.

| Patient | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|---------|---|---|---|---|---|---|---|---|
| Familial occurrence | Sporadic | Sporadic | Sporadic | Sporadic | Sporadic | Sporadic | Sporadic | Sporadic |
| ATP1A3 mutation (NM_152296.4) | c.2976_2986del, p.(Asp992del) de novo | c.2976_2986del, p.(Asp992del) de novo | c.2972_2982delins TTGGCAT CTTCATCG, p.(Tyr469_469delins PheAlaHisLeuHisLeu) de novo | c.2975_2983dup, p.(Ile994_Arg995insHisGluIle) de novo | c.2570_2572del, p.(Phe855del) de novo | c.2560_2568del, p.(Gly854_Phe856del) de novo | c.1787G>A, p.(Cys595Tyr) de novo | c.2684A>C, p.(Gln895Pro) de novo |
| Ethnicity | Japanese | Japanese | Japanese | Japanese | Israeli | Japanese | Japanese | Japanese |
| Age | 3 years 10 months | 1 year 4 months | 7 years 0 months | 5 years 9 months | 2 years 6 months (deceased at 3 years) | 10 years 9 months | 5 years 0 months | 6 years 10 months |
| Sex | Male | Female | Male | Female | Male | Female | Male | Male |
| Clinical diagnosis | Perisylvian polymicrogyria | Frontal-parietal polymicrogyria | Frontal-parietal polymicrogyria | Frontal-parietal polymicrogyria | Perisylvian polymicrogyria | Frontal-parietal polymicrogyria | Perisylvian polymicrogyria | Perisylvian polymicrogyria |
| Gestation | 37 weeks | 35 weeks | 39 weeks | 38 weeks | 35 weeks | 37 weeks | 41 weeks | 40 weeks |
| Birth length | 40.0 cm (−2.92 SD) | 40.0 cm (−2.24 SD) | 48.0 cm (−0.46 SD) | 43.6 cm (−2.38 SD) | Unknown | 49.5 cm (−1.01 SD) | Unknown | 49.0 cm (−0.00 SD) |
| Birth weight | 2152 g (−1.53 SD) | 2450 g (+0.17 SD) | 2754 g (−1.01 SD) | 2622 g (−0.82 SD) | 1820 g (10th–25th percentile) | 2928 g (+0.76 SD) | 2822 g (−0.91 SD) | 3438 g (+0.68 SD) |
| Birth head circumference | 32.0 cm (−0.48 SD) | 32.0 cm (+0.47 SD) | 31.0 cm (−1.64 SD) | 31.5 cm (−1.21 SD) | 30 cm (10th percentile) | 33.0 cm (+0.17 SD) | 31.5 cm (−1.67 SD) | 33.0 cm (−0.49 SD) |
| Facial dysmorphism | No | No | No | No | No | No | No | No |
| Other malformations | Hypo pigmented brownish hair | No | Bell-shaped narrow chest, single transverse palmar crease | No | No | No | No | No |
| Seizure onset | 30 s | Soon after birth | 10 min | 4 days | 1 hour | 2 days | No |
| Seizure types | Tonic convulsions, clonic convulsions, eye deviation, eye blinking, and twitching of the corner of the mouth | Generalized tonic-clonic seizures | Right-side dominant tonic-clonic convulsions, tonic seizures, focal or generalized clonic seizures, and autonomic seizures | Focal motor seizures and autonomic seizures | Generalized seizures starting with increased tonic in the left hand and foot, the head and eyes turn to the left, and sometimes, the body becomes tonic and clonic | Apneic spells and tonic seizures | Eye opening and nystagmus with bilateral extremity quivering and facial stiffening, followed by irregular respiration and cyanosis | No |
| EEG findings | Suppression burst at 1 month and multifocal spikes at 2 years | Frequent multifocal sharp waves with diffuse slow waves | Intercital: frequent sharp waves on T3 propagating to the left hemisphere; ictal: rhythmic activity at T3 or F4 to C4 | Bilateral frontal sharp waves with decreased background activity | Abnormal background activity and slow delta waves | Bilateral spikes with a background of slow waves | Multifocal epileptic discharges with a background of continuous diffuse slow waves | Normal at 3 months |
| Prognosis of seizures | Intractable, hourly | Seizure-free | Intractable, daily | Intractable, hourly | Intractable, partially controlled | Intractable, daily | Intractable, daily | Seizure-free |
| Development | No social smile, no head control, DQ < 10 | No social smile, no head control | No social smile, no head control, DQ = 2.5 | No social smile, no head control | No eye contact, no head control, DQ < 10 | No social smile, no head control, DQ < 10 | No social smile, no head control, DQ < 10 | No meaningful words, no rolling over |

continued on next page
of Phe-Ala-His-Leu-His-Leu into the position of the deleted p.Tyr991–Ile994 residues, and the deletion of p.Asp992) are predicted to destabilize protein folding and disrupt interactions between the α- and β-subunits.

During the reaction cycle, Na\(^+\)/K\(^+\) ATPase is autophosphorylated by ATP at p.Asp366 in the highly conserved Asp-Lys-Thr-Gly-Thr motif (16). The p.Cys596, which is close to the phosphorylation site, is involved in a hydrophobic core with many residues (p.Ile363,
Different variant distributions between AHC, RDP, CAPOS, other phenotypes, and polymicrogyria

The distribution patterns of variants for AHC, RDP, and CAPOS are similar in that they cluster within or near transmembrane domains or are located in cytoplasmic N- or P-domains, with occasional overlaps (Figs. 2A and 3A). Functionally, these variants disrupt either Na⁺/K⁺ ion binding at/near the transmembrane domain or ATP binding and subsequent phosphorylation at cytoplasmic N- or P-domains (10). Their expression may be unstable, or they may have stable expression with reduced ATPase activity (4). The CAPOS-associated p.Glu818Lys variant affects Na⁺/K⁺ ion binding and binding and subsequent phosphorylation at cytoplasmic N- or P-domains (10). In contrast, the polymicrogyria-associated variants seemed to accumulate at the β-subunit–binding site of the α-subunit, remote from ion-binding sites (10), where AHC-, RDP-, or CAPOS-associated variants rarely exist.

To statistically compare the variant distribution among different phenotypes, we collected all positions of substituted amino acids by either missense or in-frame variants for AHC, RDP, CAPOS, and other phenotypes and compared the variant distribution using the Kolmogorov-Smirnov test for AHC versus polymicrogyria, RDP versus polymicrogyria, CAPOS versus polymicrogyria, and others versus polymicrogyria. The variant distribution in polymicrogyria was significantly different from that of all other compared phenotypes (Fig. 3B).

Functional consequences of the identified ATP1A3 variants

On the basis of the structural consideration of the identified variants, we hypothesized that polymicrogyria-associated variants might impair the interaction between the β-subunits of Na⁺/K⁺ ATPase, leading to either impaired expression, trafficking, or kinetic properties of the α-subunit.

Regarding the kinetic properties of the α-subunit, a previous study reported that variants associated with both AHC and RDP affect ATPase activity (4). Thus, we performed ATPase activity assays to validate whether the detected variants affected ATPase activity. After confirming that all constructed mutant ATP1A3 complementary DNA (cDNA) plasmids had equivalent protein expression compared with the wild-type plasmids (fig. S5), we performed ATPase activity assays. ATPase activity was significantly reduced in cells transfected with ATP1A3 cDNA plasmids with p.Asp801Asn (AHC variant), p.Ile758Ser (RDP variant), or p.Cys596Tyr (polymicrogyria-associated variant) variants but was retained in cells transfected with the other polymicrogyria-associated variants (p.Gly854_Phe856del, p.Phe857del, p.Glu895Pro, and p.Asp992del) (Fig. 4A).

Next, we performed Western blotting for ATP1A3 and ATP1B1, which is one of the proteins that forms the β-subunit of Na⁺/K⁺ ATPase. We used human embryonic kidney (HEK) 293T cells cotransfected with ATP1A3 (wild-type or mutant) and ATP1B1 (wild-type) vectors to investigate protein expression with the coexistence of both α- and β-subunits. The β-subunit is glycosylated and modified to a higher molecular weight (the mature form) from the endoplasmic reticulum to the Golgi apparatus. The mature N-glycosylated β-subunit is then folded and trafficked to the cell surface with the α-subunit (2, 18). Compared with the wild-type, all polymicrogyria-associated variants had decreased expression of ATP1A3 and mature β1-subunits. This tendency was also observed in some polymicrogyria-associated variants compared with AHC- or

p.Ala599, p.Ile601, p.Val603, p.Phe745, p.Ile748, p.Val749, and p.Val752 (Fig. 2E). Thus, the p.Cys596Tyr mutation likely destabilizes protein folding and may impair catalytic activity via a structural modulation of the phosphorylation site. The FoldX-calculated free-energy change (7.88 ± 1.89 kcal mol⁻¹) caused by the p.Cys596Tyr variant is enough to explain the destabilization of the Na⁺/K⁺ ATPase complex (Fig. 2F).

Fig. 1. Bilateral perisylvian polymicrogyria associated with de novo ATP1A3 mutations. Brain MRI scans of patient 1 (A) and (B) at 1 day old, (C) and (D) at 2 years and 10 months, patient 3 ((E) and (F) at 2 months old, (G) and (H) at 8 months, patient 4 ((I) and (J) at 3 days old, (K) and (L) at 4 months, patient 5 ((M) and (N) at 2 days old, patient 6 ((O) and (P) at 2 months old, patient 7 ((Q) and (R) at 5 months old, (S) and (T) at 3 years old, patient 8 ((U) and (V) at 2 months old, and a neurologically normal control ((W) and (X) at 3 years old. All images are T2-weighted axial brain MRI, except for a T2-weighted sagittal MRI (M), a fluid-attenuated inversion recovery axial MRI (N), and an axial cranial CT (S and T). Brain MRI showed irregular atrophic changes with dilation of the subarachnoid space and lateral ventricles.
RDP-associated variants, suggesting impaired binding, folding, or trafficking of αβ-heterodimers (Fig. 4B).

We therefore performed coimmunoprecipitation analyses to validate the binding between ATP1A3 and ATP1B1. Using immunoprecipitated ATP1B1 signal intensity, we measured the relative ATP1B1 recovery rate normalized to that of the wild type; this measurement reflects binding efficiency between ATP1A3 and ATP1B1. Binding efficiency was significantly decreased in the p.Ile758Ser, p.Phe857del, and
p.Asp992del variants compared with the wild type and significantly decreased in the p.Cys596Tyr and p.Gln895Pro variants compared even with the p.Ile758Ser variant. Of these variants, p.Gln895Pro had the lowest binding efficiency. Among the total immunoprecipitated ATP1B1 (ATP1B1 bound to ATP1A3), mature β1-subunits were detected in wild-type, p.Asp801Asn, and p.Ile758Ser variants but not in any of the polymicrogyria-associated variants. This result suggests that polymicrogyria-associated variants affected both the binding to β1-subunits and the proper folding of αβ-heterodimers, although we could not exclude the possibility that decreased binding is due to the decreased expression of some ATP1A3 variants associated with polymicrogyria (Fig. 4C).

To confirm the abnormal localization of αβ-subunits because of trafficking errors, we separated total cellular components from HEK293T cells coexpressing ATP1A3 (wild type, p.Ile758Ser, or p.Gln895Pro) and ATP1B1 (wild type) into three fractions (cytosol, organelles, and plasma membrane). We chose to validate p.Ile758Ser and p.Gln895Pro variants representing non–polymicrogyria-associated and polymicrogyria-associated ones, respectively, since they showed the significantly decreased ATP1B1 recovery rate in coimmunoprecipitation analyses in each group of variants. We then evaluated ATP1A3 abundance in each fraction and measured the relative expression of mature β1-subunits in the plasma membrane fraction. In the plasma membrane, ATP1A3 expression was significantly lower in the p.Gln895Pro variant compared with the other variants. Between the p.Gln895Pro and the other variants, there was also a significant difference in the proportion of ATP1A3 abundance in each fraction. In cells expressing the p.Gln895Pro variant, ATP1A3 was significantly higher in the cytosol and lower in the plasma membrane compared with the other variants. Furthermore, in the plasma membrane fraction, immature/nascent β1-subunits were predominant in cells expressing the p.Gln895Pro variant; in contrast, in cells expressing the other variants, mature β1-subunits were significantly more common (Fig. 4D).

This result suggests a mechanism of association between the two subunits in the Golgi and their subsequent aberrant trafficking to the membrane. To validate the cellular/subcellular localizations of the α-subunits and β-subunits, we performed an immunofluorescence study using HEK293T cells cotransfected with ATP1A3 (either wild-type or the p.Gln895Pro mutant representing a polymicrogyria-associated variant) and ATP1B1, with superresolution confocal microscopy. At the single-cell level, both mutant ATP1A3 and ATP1B1 localized at the cis-Golgi network (cisternae nearest the endoplasmic reticulum) and trans-Golgi network (cisternae farthest from the endoplasmic reticulum) and at the plasma membrane, with no apparent differences in localization patterns compared with wild-type ATP1A3 and ATP1B1 (Fig. 5). Thus, p.Gln895Pro-mutant ATP1A3 is expressed in the plasma membrane. These findings retain the possibility of trafficking delay or stacking through Golgi to the plasma membrane but exclude the possibility of a complete failure of their delivery to their final destination.

To further evaluate the direct interactions between α- and β-subunits at the plasma membrane, we performed an in situ proximity ligation assay (PLA) using HEK293T cells cotransfected with ATP1A3 (either wild-type or p.Ile758Ser or p.Gln895Pro mutants) and ATP1B1 plasmids. PLA signals, derived from the interactions between α- and β-subunits, were predominantly observed at the plasma membrane. The mean PLA signal intensity of wild-type ATP1A3 and ATP1B1 expression and of p.Ile758Ser ATP1A3 and ATP1B1 expression was similar, but that of p.Gln895Pro ATP1A3 and ATP1B1 expression was apparently reduced (Fig. 6). These findings suggest that...
polymicrogyria-associated variants may have different functional impacts compared with previously known variants (such as p.Ile758Ser), likely through the decreased interaction of α- and β-subunits at the plasma membrane, possibly impairing proper Na⁺/K⁺ ATPase function but retaining ATPase activity via an as-yet unknown mechanism.

Disrupted neuronal migration in embryonic mice expressing the p.As992del variant and impaired neural activity in primary cultured neurons

To investigate the effects of ATP1A3 variants on brain development, we examined ATP1A3 overexpression in the embryonic mouse cortex using in utero electroporation. We used plasmids expressing either one of three variants (p.As992del, p.Gln895Pro, or p.Ile758Ser) or wild-type ATP1A3. We chose to validate p.As992del and p.Gln895Pro variants as the ones associated with the most severe and mildest phenotypes among our patients with polymicrogyria, respectively, while p.Ile758Ser is representing the polymicrogyria-associated variant. The four expression vectors were introduced into fetal mouse brains together with green fluorescent protein (GFP) expression vectors on embryonic day 14.5 (E14.5). Four days later, the brains were removed, and the effects on cell migration were examined. Compared with an empty GFP vector, significantly impaired
cell migration was detected only when the p.Asp992del variant was expressed. This result is consistent with our identification of this variant in the two patients with the most severe forms of polymicrogyria in our cohort. No significant differences were observed when the other variants (p.Gln895Pro or p.Ile758Ser) were expressed. Together, these findings suggest that the p.Asp992del variant disrupts cell migration during cortical development and causes defects in cortical architecture (Fig. 7, A to C).

To explore the effects of the p.Asp992del variant on neural function, we introduced wild-type and p.Asp992del ATP1A3 expression vectors, together with the calcium sensor, GCaMP6 (Green fluorescent protein – calmodulin protein 6) plasmids, by in utero electroporation.

Fig. 5. Colocalization of ATP1A3 and ATP1B1 at the cis-/trans-Golgi apparatus and plasma membrane. Through (A) to (C), the left and right panels show immunofluorescence under the cotransfection of wild-type ATP1A3 and wild-type ATP1B1 plasmids and p.Gln895Pro ATP1A3 and wild-type ATP1B1 plasmids, respectively. Scale bars, 5 μm. (A) ATP1A3 and ATP1B1 colocalizing at the cis-Golgi network in both panels (arrowhead). (B) ATP1A3 and ATP1B1 colocalizing at the trans-Golgi network in both panels (arrowhead). (C) ATP1A3 and ATP1B1 colocalizing at the plasma membrane in both panels (arrowhead). For (A) and (B), lower images are the pseudocolored maps of the product of the differences from the mean analysis (PDM maps) for the respective upper images. For (C), lower right panels are the PDM maps for the upper two images. In the PDM maps, positive correlation is indicated in yellow, negative correlation in blue, and random distribution in black.
We isolated cortical neurons from the electroporated embryonic brains, made primary cultures, and performed GCaMP imaging in DIV4. The results showed that calcium spikes were reproducibly observed to manifest neural circuit maturation in wild-type ATP1A3–introduced neurons. In contrast, the number and height of calcium spikes were significantly lower in the p.Asp992del ATP1A3–introduced neurons. This result suggests that the p.Asp992del variant may affect neural activity and impair neural function apart from cell migration in the patients with p.Asp992del variants (Fig. 7, D and E, and movie S1).

**DISCUSSION**

Here, we report eight patients with de novo ATP1A3 variants, who presented with polymicrogyria, extremely severe intellectual disability,
intractable epilepsy, and occasionally cardiac failure. Table S2 presents clinical comparisons among patients with AHC, RDP, or CAPOS, and our patients with polymicrogyria. AHC, RDP, and CAPOS are considered a continuum of clinically broad functional disorders with abrupt/paroxysmal symptoms, asymmetric anatomical distribution, presence of a trigger, and typically no morphological brain abnormalities. In contrast, our patients had relatively permanent clinical features, no phasic disease course, no obvious
trigger for disease onset, and morphological abnormalities in the brain. Thus, our patients present a new phenotype of ATP1A3 abnormalities.

In addition to the clinical phenotype, the localization of variants in ATP1A3 and their functional consequences were different between AHC/RDP/CAPOS and polymicrogyria, indicating different molecular mechanisms of pathogenesis, which may require different therapeutic strategies. Furthermore, embryonic mice with in utero electroproporation–induced overexpression of the p.Asp992del variant showed disrupted neuronal migration during cortical development, suggesting a possible association between the identified variants and defects in brain architecture.

In patients 1, 3, and 4, who had variants at the p.Tyr991–Arg995 transmembrane region, cardiac failure and progressive cerebral and cerebellar atrophy were noted. In addition, patient 6 showed progressive atrophy in the cerebellar vermis. Considering that malformations of cortical development occur during the fetal stage, the co-occurrence of postnatal brain atrophy is not particularly common but has been observed in genetic disorders such as 22q11.2 deletion syndrome (19) and Zellweger syndrome (20). Postnatal brain atrophy might therefore be associated with ATP1A3-related polymicrogyria, although more patients are needed to confirm our findings. However, we cannot exclude the possibility that other nongenetic factors, such as antiepileptic drugs and a ketogenic diet, may have led to an atrophied appearance on brain MRI scans.

The α3-subunit is highly expressed in human heart tissue. Cardiac channelopathy (21) and cardiac symptoms known as paroxysmal autonomic disturbance (22) have been reported in AHC, and left ventricular enlargement in early childhood has been noted in CAPOS (6). However, a transient cardiac failure might be a specific complication at the plasma membrane. With the decreased interaction abilities, there might also be stacking or delay of α- and β-subunits from the endoplasmic reticulum to the Golgi apparatus or some degradation of α- and β-subunits; these possibilities were suggested from the fractionation and expression analyses under cotransfection of ATP1A3 and ATP1B1 plasmids. However, these findings need to be further clarified. In addition, the decreased interaction of α- and β-subunits at the plasma membrane possibly impairs normal Na+/K+ ATPase function, although the mechanisms remain elusive (fig. S6).

The p.Gln895Pro variant, detected in the patient with the mildest clinical phenotype in our cohort, appeared to have the most severe binding and trafficking defects in our experiments. Our dominant-negative hypothesis may explain this phenomenon: There may be competition between aberrant and wild-type ATP1A3 to develop αβ-heterodimers and reach the plasma membrane. More severe trafficking errors lead to more degradation of aberrant ATP1A3; thus, more wild-type ATP1A3 can reach the plasma membrane and contribute to normal Na+/K+ ATPase functioning. A recent report on trafficking defects in αβ-heterodimers demonstrated similar experimental results and implied the possible competition of wild-type and mutant alleles, supporting our hypothesis (18).

The molecular pathogenesis of polymicrogyria remains largely unknown. Among the molecules/pathways known to cause polymicrogyria (tubulins, metabolic disorders, or the phosphatidylinositol 3-kinase–AKT–mechanistic target of rapamycin pathway), it has been proposed that mutations in genes encoding epilepsy-associated ion channel components (SCN3A, GRIN2B, and GRIN1) cause polymicrogyria by altering channel physiology during the fetal stage (26–28). This is termed “developmental channelopathy” (29), and ATP1A3 may belong to this group because of its unknown mechanisms that may relate to β-subunit malfunction (16). We found that primary cultured neurons expressing p.Asp992del variant in mice showed impaired calcium spikes leading to abnormal neuronal function. Figure 8 illustrates our understanding of the ATP1A3 gene as a cause of these ion channel diseases throughout the human life span.

From phenotypes, genotypes, and functional analyses, we demonstrate that polymicrogyria can be associated with ATP1A3 variants. Variants in ATP1A3 may be a common cause of polymicrogyria, considering our diagnostic yield of 6.5%. Clinicians should therefore be aware of both polymicrogyria and ATP1A3-related disorders because distinguishing between these diseases may be difficult using clinical symptoms only (e.g., both epilepsy in polymicrogyria and the abrupt onset of hemiplegia or autonomic symptoms in AHC can look alike).
MATERIALS AND METHODS

Participants
In total, 124 families with polymicrogyria were recruited to the study. Experimental protocols were approved by the Committee for Ethical Issues at Yamagata University Faculty of Medicine, Showa University School of Medicine, and Yokohama City University School of Medicine. Written informed consent was obtained from all individuals or their parents. Clinical information was collected from medical records.

Experimental animals
All animals were treated in accordance with the Tokyo Metropolitan Institute of Medical Science Animals Care and Use Committee guidelines. Pregnant ICR (Institute of Cancer Research) mice were purchased from Japan SLC Inc. (Hamamatsu, Japan).

Whole-exome sequencing
As previously described, 114 of 124 families were sequenced at Yokohama City University (30). Among these 114 families, trio-based or patient-only whole-exome sequencing was performed in 54 families and 60 patients, respectively. For these 114 families, the mean depth of coverage for whole-exome sequencing was 68.98×, and 96.17% of the total coding sequence of RefSeq genes was covered with a depth of 10× reads or more, on average (88.3 to 98.1%). The other three and seven families in this study were analyzed at Hamamatsu University School of Medicine (including patient 7) and RIKEN Center for Integrative Medical Sciences (including patient 1), respectively. All variants were confirmed by Sanger sequencing.

Protein structure modeling
The structure of the human Na+/K+ ATPase α3-subunit is not available in the PDB; therefore, we used the Phyre2 program to search for a homologous structure using its amino acid sequence (RefSeq NM_152296.4) (14). Next, we used FoldX (ver.3.0) (31, 32) to calculate free-energy changes caused by the identified mutations, using the crystal structure of S. acanthias Na+/K+ ATPase (PDB code: 2ZXE) as a homologous template (listed by Phyre2). The calculation was repeated five times, and data were presented as the means ± SD. The modeled structures were drawn using PyMOL (www.pymol.org).

Vectors and transfection procedures
Human ATP1A3 (ORH26659) and ATP1B1 (FHC02644) clones were purchased from Promega (Madison, WI, USA). Human ATP1A3 cDNA was introduced into a pcDNA3.1/myc-His C vector (Invitrogen, Carlsbad, CA, USA) to express C-terminal myc-His–tagged ATP1A3. Site-directed mutagenesis using the KOD-Plus-Mutagenesis Kit (Toyobo, Osaka, Japan) allowed the generation of mutant ATP1A3 vectors (p.Asp992del, p.Phe857del, p.Gly854_Phe856del, p.Cys596Tyr, p.Gln895Pro, p.Ile758Ser, and p.Asp801Asn). For the animal experiments, wild-type and mutated ATP1A3 expression plasmids for in utero electroporation were constructed using PB CAG-EGFP vectors (33). Briefly, CAG-ATP1A3 variant vectors were assembled in a pUC57-mini plasmid backbone (Genscript Biotech Corp., Piscataway, NJ, USA) using standard restriction- and ligation-based cloning. We drove ATP1A3 expression with the strong eukaryotic CAG promoter. The inserts of ATP1A3 variants were cloned into Eco RI/Sma I enzyme sites on pB CAG-EGFP using Ligation high Ver.2 (Toyobo). For the ATPase assays, COS-7 cells were transfected with ATP1A3 expression plasmids using ViaFect Transfection Reagent (Promega). For the rest of the analyses, HEK293T cells were transfected with ATP1A3 and/or ATP1B1 expression plasmids using either ViaFect Transfection Reagent or polyethylenimine (Polysciences Inc., Warrington, PA, USA) according to the manufacturer’s protocol.

Western blotting
Capillary electrophoretic Western blotting was performed on the Wes system (ProteinSimple, San Jose, CA, USA) using a 12- to 230-kDa
separation module according to the manufacturer’s protocol for size separation. Myc-tagged ATP1A3, HaloTag-fused ATP1B1, and β-actin were detected using mouse anti–Myc-tag monoclonal antibody (mAb) [MBL(MEDICAL & BIOLOGICAL LABORATORIES), Nagoya, Japan], rabbit anti-HaloTag polyclonal antibody (pAb) (Promega), mouse anti-HaloTag mAb (Promega), and mouse anti–β-actin (ab6276; Abcam, Cambridge, UK), respectively. To prepare cell lysate for ATP1A3 detection, we did not boil the lysate but stored it at 4°C overnight with urea at its final concentration of 4 M.

ATP1A3 protein blotting
HEK293T cells were transfected with ATP1A3 (wild type or mutant) cDNA vectors. Forty-eight hours after transfection, the cells were lysed with radioimmunoprecipitation assay buffer and subjected to Western blotting on the Wes system to detect ATP1A3 and β-actin signals, using anti–Myc-tag and anti–β-actin antibodies.

ATPase assay
COS-7 cells expressing wild-type or mutant alleles of ATP1A3 were assessed in the ATPase assay as described previously (4). Briefly, synthesized adenosine 5′-diphosphate (ADP) by ATPase reaction was measured and subjected to Western blotting to detect ATP1A3 and β-actin signals, using anti–Myc-tag and anti–β-actin antibodies.

ATP1A3 and N-glycosylated β1-subunit protein blotting
Lysate from HEK293T cells cotransfected with ATP1A3 (wild type or mutant) and ATP1B1 (wild type) cDNA vectors was collected 48 hours after transfection and was subjected to Western blotting to detect ATP1A3, ATP1B1, and β-actin signals using anti–Myc-tag, anti-HaloTag, and anti–β-actin antibodies. Relative ATP1A3 expression was calculated as the band intensity of ATP1A3 normalized to that of β-actin and to wild-type ATP1A3. For ATP1B1, the molecular weight of nascent, immature (stayed at the endoplasmic reticulum), and mature (in the Golgi apparatus or trafficked to the cell surface) HaloTag-fused ATP1B1 were predicted to be around 60, 80, and 100 kDa in size, respectively. We calculated the relative mature β1-subunit expression by dividing the signal intensity of mature β1-subunits by that of total β1-subunits and then normalized it to the wild type. The denatured lysate was then treated with PNGase F (N-Zyme Scientifics, Doylestown, PA, USA) for 2 hours at 37°C to cleave the N-linked oligosaccharides, to ensure that the higher molecular signals disappeared.

Immunoprecipitation
For the coimmunoprecipitation assay, wild-type or mutant myc-tagged ATP1A3 vectors and HaloTag-fused ATP1B1 vectors were cotransfected into HEK293T cells. At 48 hours after transfection, cells were lysed with Mammalian Lysis Buffer (Promega) containing Protease Inhibitor Cocktail (Promega) and PhosSTOP (Roche Diagnostics, Basel, Switzerland). The lysate was incubated with 2 μg of anti–Myc-tag mAb (MBL) at 4°C overnight and with Dynabeads Protein G (Thermo Fisher Scientific, Waltham, MA, USA) at 4°C for 2 hours. After washing with phosphate-buffered saline (PBS), the beads were resuspended with 1% SDS buffer. Inputs and immunoprecipitates were subjected to Western blotting to detect ATP1A3 and ATP1B1 using anti–Myc-tag and anti-HaloTag antibodies. Because an excess of both myc-tagged ATP1A3 and HaloTag-fused ATP1B1 was confirmed in the flow-through after immunoprecipitation by Western blotting, and equivalent amounts of anti–Myc-tag mAb were added to the lysates for immunoprecipitation, we measured the band intensities of HaloTag signals from each immunoprecipitate. We then calculated the binding efficiency between mutant ATP1A3 and wild-type ATP1B1 as the HaloTag signal intensity of the particular immunoprecipitate divided by that of wild-type ATP1A3 and wild-type ATP1B1.

ATP1A3 and ATP1B1 localization analysis in HEK293T cells
HEK293T cells were cotransfected with ATP1A3 (wild-type, p.Ile758Ser, or p.Gln895Pro) and ATP1B1 (wild-type) cDNA vectors and collected 48 hours after transfection. Using the Plasma Membrane Protein Extraction Kit (101Bio, Palo Alto, CA, USA), we separated the total cellular components into three fractions: cytosol, organelles, and plasma membrane. This was conducted according to the manufacturer’s instructions, and the three fractions were then subjected to Western blotting to detect ATP1A3 and ATP1B1 signals using anti–Myc-tag and anti-HaloTag antibodies. We also used mouse anti–β-actin antibody, anti-GM130 (anti-Golgi subfamily A member 2) antibody [EP892Y, cis-Golgi Marker (ab52649; Abcam)], and ATP1A3 (middle) rabbit pAb (28030-1-AP, Proteintech, Rosemont, IL, USA) as marker antibodies for the respective cellular fractions. We calculated the relative ATP1A3 expression of p.Ile758Ser or p.Gln895Pro compared with wild-type variants in the cytosol, organelles, and plasma membrane as follows: The band intensities of ATP1A3 in either fraction were divided by that of either β-actin, GM130, or endogenous ATP1A3 and normalized to that of wild type. We also calculated the relative ATP1A3 expression in each of the three fractions as the band intensities of ATP1A3 in a fraction divided by that of the sum of all three fractions. In addition, we calculated the relative expression of differently N-glycosylated ATP1B1 in the plasma membrane fraction as the band intensities of mature β1-subunits divided by the band intensities of the sum of all β1-subunits (nascent, immature, and mature) in the plasma membrane fraction.

Immunofluorescence study
Forty-eight hours after transfection, HEK293T cells on glass coverslips were fixed with 4% paraformaldehyde (PFA)/PBS for 10 min. After being washed twice with PBS-glycine, the cells were permeabilized with 0.1% Triton X/PBS for 10 min. The cells were then blocked with 10% normal goat serum (Jackson ImmunoResearch Laboratories, West Grove, PA, USA)/0.05% Tween 20/PBS before immunolabeling was performed using anti-HaloTag pAb [1:500, with goat anti-rabbit immunoglobulin G (IgG) (H + L) secondary antibody; Alexa Fluor 546 conjugate, Thermo Fisher Scientific; 1:500] and mouse anti–Myc-tag mAb [1:1000, with goat anti-mouse IgG (H + L) highly cross-adsorbed secondary antibody; Alexa Fluor 647, Invitrogen; 1:1000], for plasma membrane evaluation. For Golgi apparatus evaluation, we performed immunolabeling with mouse anti–Myc-tag mAb [1:1000, with either goat anti-mouse IgG2b cross-adsorbed secondary antibody; Alexa Fluor 647, Invitrogen; 1:1000; or goat anti-mouse IgG (H + L) highly cross-adsorbed secondary antibody; Alexa Fluor 488, Invitrogen; 1:1000] as a cis-Golgi marker, and golgin-97 (D8P2K) rabbit mAb
Microscopy and image analysis

Conventional and super-resolution confocal images were acquired using the TCS SP8 System (Leica, Wetzlar, Germany) with 20×, 63×, and 100× objective lenses (HC PL APO 20×/0.75 DRY, 63×/1.40 OIL, and 100×/1.40 OIL, respectively). Excitation was provided by a white-light laser, and the fluorescent signal was acquired using a GaAsP hybrid detector system (Leica). For the superresolution microscopy, we set the pinhole at a diameter of 0.5 to 0.75 Airy units, and acquired images were deconvoluted using Huygens software (Scientific Volume Imaging, Hilversum, Netherlands), if necessary. Images were quantified and arranged using ImageJ 1.53 software (National Institutes of Health, Bethesda, MD, USA) and Photoshop 12.0 (Adobe, San Jose, CA, USA).

In situ PLA

We performed the in situ PLA using Duolink In Situ PLA Fluorescence (Sigma-Aldrich) following the manufacturer’s protocol. Forty-eight hours after transfection, HEK293T cells on an eight-well slide chamber were fixed with 4% PFA/PBS for 10 min and permeabilized with 0.1% Triton X/PBS for 10 min before starting the Duolink procedure. We used mouse anti–Myc-tag mAb (1:1000) and anti-HaloTag pAb (1:500) as primary antibodies, anti-mouse PLUS and anti-rabbit MINUS as Duolink PLA probes, and Detection Reagents Red for signal detection. We calculated the mean intensity of spec- kled signals at each of the specified regions of interest (ROIs) where both HaloTag-fused ATP1B1 and phallolidin signals were obtained (the numbers of ROIs were 691 for wild-type ATP1A3/ATP1B1, 479 for p.Ile758Ser mutant ATP1A3/ATP1B1, and 449 for p.Gln895Pro mutant ATP1A3/ATP1B1, respectively).

GCaMP imaging of primary cultured neurons overexpressed with wild-type or p.Asp992del variant of ATP1A3

GCaMP6 and red fluorescent protein plasmids were electroporated together with either wild-type or p.Asp992del ATP1A3 expression vectors at E14.5. The electroporated embryonic brains were dissected at E16.5, and dorsal cortices were excised and dissociated with papain. Cells were cultured in the polyethyleneimine-coated glass-bottom dishes in minimum essential medium–base culture medium. At DIV 4, GCaMP signals of cultured neurons were monitored using spinning disk confocal scanner unit CSU-W1 (YOKOGAWA, Tokyo, Japan). For each recording, 1200 images were captured every 400 ms. Intensities of fluorescent signals were analyzed by IQ3 Cell Imaging Software (Andor, Belfast, Northern Ireland). Only peak that have an intensity of >1.5 times the average fluorescent signal value was considered valid. Two independent in utero electroporation experiments were performed, and six and four electroporated brains from wild-type and p.Asp992del variant mice were collected, respectively. We acquired the calcium imaging data from N = 9 or more observations (wild type; N = 11, p.Asp992del; N = 9) for statistical processing.

Statistical analysis

All statistical analyses were performed using GraphPad Prism8 (GraphPad Software, San Diego, CA, USA). For the ATP1A3 expression analyses, ATPase assays, ATP1A3, N-glycosylated β1-subunit protein blotting, and immunoprecipitation, statistical differences were analyzed using one-way analysis of variance (ANOVA) with post hoc Dunnett’s test for multiple comparisons. For the ATP1A3 and ATP1B1 localization analysis, we used two-way repeated-measure ANOVA with post hoc Tukey’s test for multiple comparisons. To compare ATP1A3 variant distribution among phenotypes, we first collected all positions of substituted amino acids by either missense or in-frame variants for AHC, RDP, CAPOS, and other phenotypes (including those who manifested some of the symptoms for AHC, RDP, or CAPOS but were unable to be specifically diagnosed with them) from the Human Gene Mutation Database (https://portal.biobase-international.com/cgi-bin/portal/login.cgi; as of 25 August 2020). For an amino acid where multiple patients (multiple reports) had substitutions, we counted cumulatively. We then performed the Kolmogorov-Smirnov test to compare the variant distribution tendencies of AHC versus polymicrogyria, RDP versus polymicrogyria, CAPOS versus polymicrogyria, and others versus polymicrogyria. A value of P < 0.05 was considered significant, except for the Kolmogorov-Smirnov test, which used P < 0.0125 (after Bonferroni correction) because multiple tests were performed. For the mouse experiments, statistical differences were analyzed using the Student’s t test.

abdominal cavity to allow the embryos to continue their development. The brains of the electroporated embryos were harvested at E18.5, fixed in 4% PFA/PBS for 20 hours, and embedded with OCT (optimal cutting temperature) compound after sucrose substitution. Frozen sections were prepared from each brain at a thickness of 20 μm. After 4',6-diamidino-2-phenylindole staining, images were acquired using an LSM 780 confocal laser microscope (Zeiss, Jena, Germany). The GFP-positive migrating cells in each image were counted using ImageJ software. Two brains (for GFP, wild type, and the p.Ile758Ser variant) or four brains (p.Asp992del and p.Gln895Pro variants) were used for each model. Between two and four sections from each brain were analyzed (N = 5 for p.Ile758Ser, N = 6 for wild type and p.Gln895Pro, N = 7 for GFP, and N = 8 for p.Asp992del).
We thank all the participants for cooperation in this research. We acknowledge the contributions of K. Ishii, C. Ohno, H. Okada, H. Watanabe, D. Saisho, and K. Takeuchi from Department of Pediatrics, Showa University School of Medicine; and M. Kasai, K. Nakajima, H. Okada, RP58 regulates the multipolar-bipolar transition of newborn neurons in the developing cerebral cortex. Cell Rep. 3, 458–471 (2013).

J. Uchitel, A. Helseth, L. Prange, M. McLean, R. Ghusayni, M. Sachdev, A. Hunanyan, M. Schlesinger-Massart, A. J. Lewelt, S. P. Reyna, K. J. Swoboda, Alternating hemiplegia of childhood: Early characteristics and evolution of a neurodevelopmental syndrome. Pediatrics 123, e534–e541 (2009).

Acknowledgments: We thank all the participants for cooperation in this research. We are grateful to K. Takabe, N. Watanabe, S. Sugimoto, and S. Nakamura from the Department of Human Genetics, Yokohama City University Graduate School of Medicine; K. Tanaka from the Department of Pediatrics, Yamagata University Faculty of Medicine; M. Ogawa from the Department of Pediatrics, Showa University School of Medicine; and M. Yokouchi and F. Sakurada from Gene Regulation Research, Nara Institute of Science and Technology for technical assistance. We also thank A. Ryo from Department of Microbiology, Yokohama City University Graduate School of Medicine for invaluable advice toward the functional study and B. Gardner from Edanz Group (https://en-author-services.edanzgroup.com/ac) for editing a draft of this manuscript. Funding: This work was supported by the Japan Agency for Medical Research and Development (AMED) under grant numbers JP20ek0109280, JP20dm0107090, JP20ek0109301, JP20ek0109348, and JP20ek0205012 (N.Ma.); JP19ek0109297 (H.S. and M.K.); JP19k0201069 (M.K.); and JP20dm0307028, JP20km0405214, and JP20ek0109381 (A.T.) and JP18ek0109288h00021 (T.Ma.); JSPS KAKENHI under grant numbers JP17H01539 (N.Ma.), JP16H05160 (H.S.), JP19H03621 (N.Mi.), JP17K10080 (S.M.), JP17H05621 (T.Ma.), JP19K16921 (E.K.), JP20H03270 (C.O.-M.), and JP16K09975 (M.K.); Grant-in-Aid for Scientific Research on Innovative Areas “Interplay of developmental clock and extracellular environment in brain formation” under grant number JP19H04785 (C.O.-M.); and intramural research grants for Neurological and Psychiatric Disorders of NCNP from the Ministry of Health, Labour and Welfare under grant numbers 30-6 (N.Ma. and M.K.) and 30-7 (N.Ma.), the Naito Foundation (C.O.-M.), the Takeda Science Foundation (N.Mi., H.S., M.N., C.O.-M., and N.Ma.), and the Suntory Foundation for Life Sciences Bioorganic Research Institute (T.Ma.).

Author contributions: S.M., M.K., T.K., C.O.-M., and N.Ma. designed the study. M.K., Y.So., M.Sas., H.To., T.Ha., Y.Sa., S.O., Y.Na., Mazumi Miura, Y.Ni., K.N., H.A., H.T., T.Y., N.K., A.H., M.F., H.I., G.M., N.E., and Masaki Miura performed the clinical analyses. S.M., E.K., T.Ma., H.Take., H.D., K.S., A.Y., M.N., T.Hi., K.Haman., M.Sat., T.Miy., F.M., T.T., T.Miz., N.Mi., A.S., H.S., H.Taka., and F.T. performed the genetic and functional analyses. K.Hamad. and K.O. performed the structural analyses. T.K. and C.O.-M. performed the mouse experiments and analyses. N.Ma. supervised all aspects of the study. S.M., M.K., K.Hamad., K.O., H.Take., T.K., C.O.-M., T.Ma., and N.Ma. wrote the paper. All authors reviewed the paper and approved the final manuscript before submission. Competing interests: The authors declare that they have no competing interests.

Data and materials availability: All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials. Additional data related to this paper may be requested from the authors.

Submitted 9 June 2020
Accepted 4 February 2021
Published 24 March 2021
10.1126/sciadv.abd2368

Citation: S. Miyatake, M. Kato, T. Kumamoto, T. Hirose, E. Koshimizu, T. Matsui, H. Takeuchi, H. Doi, K. Hamada, M. Nakashima, K. Sasaki, A. Yamashita, A. Takata, K. Hanamaka, M. Satoh, T. Miyama, Y. Sonoda, M. Sasazuki, H. Torisu, T. Hara, Y. Sakai, Y. Noguchi, M. Miura, Y. Nishimura, K. Nakamura, H. Asai, N. Hinokuma, F. Miya, T. Tsuchida, M. Togawa, Y. Ikeda, N. Kimura, K. Amemiya, A. Horino, M. Fukuo, H. Ikeda, G. Merhav, N. Ekhlisvitch, M. Miura, T. Mizuguchi, H. Miyake, A. Suzuki, S. Ohga, H. Saito, H. Takahashi, F. Tanaka, K. Ogata, C. Ohnaka-Maruyama, N. Matsumoto, De novo ATP1A3 variants cause polymicrogyria. Sci. Adv. 7, eabd2368 (2021).
De novo ATP1A3 variants cause polymicrogyria
Satoko Miyatake, Mitsuhiro Kato, Takuma Kumamoto, Tomonori Hirose, Eriko Koshimizu, Takaaki Matsui, Hideyuki Takeuchi, Hiroshi Doi, Keisuke Hamada, Mitsuko Nakashima, Kazunori Sasaki, Akio Yamashita, Atsushi Takata, Kohei Hamanaka, Mai Satoh, Takabumi Miyama, Yuri Sonoda, Momoko Sasazuki, Hiroyuki Torisu, Toshio Hara, Yasunari Sakai, Yushi Noguchi, Mazumi Miura, Yoko Nishimura, Kazuyuki Nakamura, Hideyuki Asai, Nodoka Hinokuma, Fuyuki Miya, Tatsuhiko Tsunoda, Masami Togawa, Yukihiro Ikeda, Nobusuke Kimura, Kaoru Amemiya, Asako Horino, Masataka Fukuoka, Hiroko Ikeda, Goni Mermel, Nina Ekhilevitch, Masaki Miura, Takeshi Mizuguchi, Noriko Miyake, Atsushi Suzuki, Shouichi Ohga, Hirotomu Saito, Hidehisa Takahashi, Fumiaki Tanaka, Kazuhiro Ogata, Chiaki Ohtaka-Maruyama and Naomichi Matsumoto

Sci Adv 7 (13), eabd2368.
DOI: 10.1126/sciadv.abd2368