Brain monoamine vesicular transport disease caused by homozygous SLC18A2 variants: a study in 42 affected individuals


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Abstract

Purpose: Brain monoamine vesicular transport disease is an infantile-onset movement disorder that mimics cerebral palsy. In 2013, the homozygous SLC18A2 variant, p.Pro387Leu, was first reported as a cause of this rare disorder, and dopamine agonists were efficient for treating affected individuals from one large family. To date, only six variants have been reported. Here, we evaluated genotype–phenotype correlations in individuals with biallelic SLC18A2 variants.

Methods: Forty-two affected individuals with homozygous SLC18A2 variant alleles were identified. We evaluated genotype–phenotype correlations and the missense variants in the affected individuals, based on the structural modeling of rat vesicular monoamine transporter type 2 encoded by Slc18a2, with cytoplasm- and lumen-facing conformations. A Caenorhabditis elegans model was created for functional studies.

Results: Nineteen homozygous SLC18A2 variants, including three recurrent variants, were identified using exome sequencing. The affected individuals typically showed global developmental delay, hypotonia, dystonia, oculogyric crisis, and autonomic nervous involvement (temperature dysregulation/sweating, hypersalivation, and gastrointestinal dysmotility). Among the 58 affected individuals described to date, 16 (28%) died before the age of 13 years. Nine of the 17 patients with p.Pro237His died,
whereas all 14 patients with p.Pro387Leu survived. Although a dopamine receptor agonist mildly improved the disease symptoms in 17 of 21 patients (81%), some affected individuals with p.Ile43Phe and p.Pro387Leu showed milder phenotypes and, presented prolonged survival, even without treatment. The *C. elegans* model showed behavioral abnormalities.

**Conclusions:** These data expand the phenotypic and genotypic spectra of *SLC18A2*-related disorders.

**Keywords:** solute carrier family 18 member A2, brain vesicular monoamine transporter type 2, brain monoamine vesicular transport disease, dopamine agonist, Parkinsonism, dystonia

**Abbreviations:** SLC18A2: solute carrier family 18 member A2; VMAT2: brain vesicular monoamine transporter type 2; rVMAT2: rat VMAT2; TM1: transmembrane helix 1

**INTRODUCTION**

Monoamine neurotransmitter disorders are rare heterogeneous neurological disorders
mostly presenting during early life.\textsuperscript{1,2} Many neurotransmitter disorders resemble the phenotypes of other neurological disorders (e.g., cerebral palsy and hypoxic ischemic encephalopathy) and are thus sometimes misdiagnosed.\textsuperscript{3} When dyskinetic movements occur in combination with autonomic dysregulation, there is a possibility of a neurotransmitter disease, and hence, genetic diagnosis should be considered. Biallelic loss-of-function (LoF) variants in \textit{SLC6A3} (MIM*126455), which encodes a dopamine transporter, cause infantile-onset Parkinsonism-dystonia 1 (PKDYS1; MIM# 613135), known as dopamine transporter deficiency syndrome (DATS), the first monoamine transportopathy to be described.\textsuperscript{4,5} Brain vesicular monoamine transporter type 2 (VMAT2), encoded by the solute carrier family 18 member A2 gene (\textit{SLC18A2}, MIM*193001), facilitates dopamine and serotonin loading into synaptic vesicles for their transport to the cell membrane and the subsequent release.\textsuperscript{6} Biallelic dysfunction of \textit{SLC18A2} causes brain monoamine vesicular transport disease (infantile-onset Parkinsonism-dystonia 2 [PKDYS2]; MIM# 618049).\textsuperscript{7} Heterozygous \textit{Slc18a2}-knockout mice express half the amount of VMAT2 in the brain compared with that in the wild-type mice, and homozygous knockout mice do not express VMAT2 and have poor postnatal viability.\textsuperscript{6,8} A homozygous \textit{SLC18A2} variant (c.1160C>T p.Pro387Leu) was first identified in a single large consanguineous
family, wherein eight individuals were affected. Subsequently, a small number of cases with other SLC18A2 variants were reported. To date, six disease-causing variants in SLC18A2 have been reported in seven families, involving 16 affected individuals; among them, only six cases have been described in detail. In addition, following the first report, each study has described only a single pedigree with one disease-causing variant and thus comprehensive genetic and clinical aspects of the trait remain elusive.

Here, we evaluated 19 homozygous SLC18A2 variants affecting 42 individuals in 26 families. Together with functional studies, our data could better elucidate the molecular and phenotypic spectra of the VMAT2 aberration.

MATERIALS AND METHODS

Genetic and clinical investigations

We enrolled 42 affected individuals who were newly identified with disease-causing homozygous SLC18A2 variants through exome/genome sequencing (ES/GS), data sharing with international collaborators and using GeneMatcher. The study was approved by the appropriate Institutional Review Board. Written informed consent to perform genetic studies and publish clinical data, including photographs, was obtained from the parents of all patients. The clinical features of patients were retrospectively
investigated.

Genomic DNA was isolated from peripheral blood leukocytes using standard procedures, and ES/GS was performed on samples from all affected individuals and in some cases, on samples from their parents. The Genome Aggregation Database (gnomAD)\textsuperscript{16} and the UK Biobank database\textsuperscript{17} was used to select rare variants that were either absent or present at extremely low frequencies in public databases. NM\_003054.6 was used as the coding reference sequence for the \textit{SLC18A2} gene. The identified rare disease-causing variants in \textit{SLC18A2} were confirmed using Sanger sequencing of amplicons. The disease causality of variants was evaluated using \textit{in silico} prediction scores.

\textbf{Structural analysis based on homology models}

Molecular structural analyses of the mutant proteins were performed based on the conceptual translation of the detected \textit{SLC18A2} variants. Homology models of rat brain vesicular monoamine transporter type 2 (rVMAT2) in the cytoplasm- and lumen-facing conformations (accession numbers PM0078823 and PM0080553, respectively) were obtained from the Protein Model DataBase.\textsuperscript{18} A model structure of human VMAT2, predicted using AlphaFold (AF-Q05940-F1-model\_v2.pdb), was obtained from the
AlphaFold Protein Structure Database. Structural considerations and figure preparation were performed using PyMOL (Schrodinger, Inc., New York, NY, USA).

**SLC18A2 loss-of-function Caenorhabditis elegans model**

Worm models of genetic diseases are useful for mechanistic studies of disease-related gene function and for drug repurposing screens. The treatments for patients with VMAT variants present mixed efficacy, and mutations in the *C. elegans SLC18A2* homolog *cat-1* remain largely uncharacterized. Therefore, we attempted to develop a suitable *C. elegans* model to identify novel candidate treatments *in vivo*. CRISPR-Cas9 was used to generate a large putative LoF mutant, *cat-1*(syb4974). Automated quantitative phenotyping was used to evaluate a consistent multidimensional behavioral phenotype of the disease model in comparison with that of the wild-type strain, N2.

**Generation of mutant Caenorhabditis elegans mutant**

The mutant was designed by SunyBiotech using N2 background as a reference. CRISPR guide RNA was designed to target a large deletion (4508 bp), starting close to the start codon and excising several exons from the gene to give high confidence of a putative LoF allele. Deletions were confirmed using polymerase chain reaction.
**Worm preparation**

All strains were cultured on Nematode Growth Medium at 20°C and fed *Escherichia coli* (OP50) following a standard procedure. For imaging, synchronized populations of young adult worms were cultured by bleaching unsynchronized gravid adults and allowing L1 diapause progeny to develop for 2.5 days at 20°C (detailed protocol: https://dx.doi.org/10.17504/protocols.io.2bzgap6). On the day of imaging, young adults were washed in M9 (detailed protocol: https://dx.doi.org/10.17504/protocols.io.bfqbjmsn), transferred onto imaging plates (3 worms/well) using a COPAS 500 Flow Pilot (detailed protocol: https://dx.doi.org/10.17504/protocols.io.bfc9jiz6), and incubated at 20°C for 3.5 h. The plates were transferred onto a multi-camera tracker for another 30 min for habituation before imaging (detailed protocol: https://dx.doi.org/10.17504/protocols.io.bsicncaw).

**Image acquisition, processing, and feature extraction.**

Videos were acquired and processed following previously described methods. Briefly, videos were acquired in a room with a nominal temperature of 20°C at 25 frames/s at a resolution of 12.4 µm/px. Three videos were recorded sequentially: a 5-min
pre-stimulus video; a 6-min blue-light recording with three 10-s blue light pulses starting at 60, 160, and 260 s; and a 5-min post-stimulus recording.

The videos were segmented and tracked using Tierpsy Tracker. After segmentation and skeletonization, a manual threshold was applied to filter skeletonized objects—likely to be non-worms from feature extraction—that did not meet the following criteria: 200–2000 µm in length and, 20–500 µm in width. In addition, the Tierpsy Tracker viewer was used to mark wells with visible contamination, agar damage, or excess liquid as “bad,” and these wells were excluded from further analysis.

Following tracking, we extracted a pre-defined set of 3076 behavioral features for each well in each of the three videos (pre-stimulus, blue light, and post-stimulus). The extraction of behavioral features was performed on a per-track basis, and the features were then averaged across tracks to produce a single feature vector for each well. Significant differences between the pre-stimulus, post-stimulus, and blue-light behavioral feature sets extracted from the LoF mutants compared with the N2 reference strain were calculated using block permutation t-tests (https://github.com/Tierpsy/tierpsy-tools-python/blob/master/tierpsytools/analysis/statistical_tests.py). Python (version 3.8.5) was used to perform the analysis using $n = 1000000$ permutations that were randomly shuffled within, but not between, the
independent days of image acquisition to control for daily variations in the experiments. The \( p \)-values were then corrected for multiple comparisons using the Benjamini–Hochberg Procedure\textsuperscript{27} to control the false discovery rate at 5%.

Pharyngeal pumping assay

Pharyngeal pumps per minute (ppm) of the \textit{C. elegans} strains were determined by counting grinder movements by eye over a 20-s period using a stereomicroscope (detailed protocol: dx.doi.org/10.17504/protocols.io.b3hiqj4e), \( n = 120 \) worms. Grinder movements of a single worm were counted three times, and the results were recorded as an average of these values. Significant differences in ppm between the N2 reference strain and \textit{cat-1} (syb4974) mutants were calculated using block permutation \( t \)-tests with \( n = 10000 \) permutations.

RESULTS

Genetic and clinical findings in the affected individuals

Four nonsense variants, five frameshift variants, one splice site variant, and nine missense variants (all homozygous) were identified in \textit{SLC18A2} in 27 unrelated families involving 42 affected individuals (\textbf{Figure 1a}). Homozygous variants simply facilitated
the establishment of genotype–phenotype correlations. Of the variants identified, 17
were novel, whereas two missense variants (NM_003054.6: c.710C>A p.Pro237His and
c.1160C>T p.Pro387Leu) were recurrent, accounting for 43% of the cases (N = 18/42)
described herein. The most common recurrent missense variant, p.Pro237His, was
identified in 12 affected individuals from six families. This variant had previously been
identified in five patients from three families.9-11 The second most common variant in
this cohort was p.Pro387Leu, which was identified in six affected individuals from three
families and has also been previously described in different ethnicities. The novel
nonsense variant p.Tyr81* was identified in three affected individuals from two
unrelated families. All predicted disease-causing SLC18A2 variants identified in this
study were either ultra-rare or absent in multiple population variant databases
(Supplementary Table 1); however, p.Pro237His was relatively frequent in the general
population, and was found in six of 251,386 alleles (0.000024) in gnomAD and 35 of
537,496 alleles (0.000065) in the UK Biobank database as the heterozygous state.16,17
All nonsense and frameshift variants were expected to result in nonsense-mediated
mRNA decay. All missense variants had Phred-scaled Combined Annotation Dependent
Depletion scores greater than 25 (Supplementary Table 1). Two individuals had other
candidate variants; Patient 41 with the homozygous SLC18A2 variant (c.282delA
p.Asp95Thrfs*2) was described previously,\textsuperscript{28} and had potentially multiple molecular diagnoses due to compound heterozygous \textit{DDX47} variants (c.[22G>T];[319G>G]) and a homozygous \textit{SLC13A5} variant (c.1444A>G). Patient 39 with the homozygous \textit{SLC18A2} variant (c.282delA) p.Asp95Thrfs*2) had another candidate variant in the \textit{TOR1A} gene (c.836T>C). p.Met279Thr).

Sixteen of the 58 individuals (28\%) described to date (42 individuals in this study and 16 individuals who were previously reported, with homozygous variants) died during childhood (age range: 9 months to 13 years; median: 5.5 years) due to pulmonary complications, sudden cardiorespiratory arrest, or high fever with/without seizures. The clinical features of patients with biallelic disease-causing \textit{SLC18A2} variants detected in the previous and present studies are summarized in Table 1 and fully described in \textbf{Supplementary Table 2}. Except Patient 5, who was prematurely born with a birthweight of 1.3 kg, most affected individuals were neurologically normal at birth with no perinatal problems. A few weeks to a few months after birth, the affected individuals began to manifest muscular hypotonia, feeding difficulties, and global developmental delay. Most individuals presented global developmental delay (100\%, 57/57), truncal hypotonia (96\%, 53/55), dystonia (94\%, 51/54), and parkinsonism (73\%, 36/49).
however, the severity varied. Oculogyric crisis is a critical sign of this genetic disorder (88%, 44/50). Temperature instability or excessive sweating was observed in 71% (32/45) of the patients. Gastrointestinal problems including dysphagia, hypersalivation, and constipation were frequently observed in 63% (32/43), 78% (35/45), and 69% (18/26) of the patients, respectively, and several patients required supplemental nasogastric feeding or gastrostomy. Epilepsy or seizures occurred in 40% (18/45) and other paroxysmal movements were observed in 58% (28/48) of the patients. However, the electroencephalogram (EEG) was normal or at least did not correlate well with seizures or other paroxysmal movements. Several patients showed intentional tremor or ataxia, although we could not obtain sufficient information for most patients.

The brain images were typically normal (40%, 15/37), although subtle changes were occasionally observed (e.g., corpus callosum hypoplasia in 14% [5/37], non-specific white matter abnormalities in 27% [10/37], or cerebral atrophy/cortical volume loss/mild ventricle enlargement in 30% [11/37] of the patients; Table 1, Supplementary figure 1). Cerebrospinal fluid (CSF) neurotransmitter analysis was performed in four patients, and homovanillic acid (HVA) and 5-hydroxyindoleacetic acid (5-HIAA) levels were within normal limits (Supplementary Table 3).

In total, 80% (33/41) of the patients were non-verbal and most of them were
non-ambulatory. Fourteen affected individuals with nonsense, frameshift, and splice site
variants were mostly non-verbal and in a bed-bound state, and had more severe
symptoms than those with missense variants (e.g. Figure 1b; photographs of Patients 30,
31, 34, 40, and 42 with truncating variants). Notably, differences in disease severity
were observed among the affected individuals with missense variants, wherein the
patients with p.Pro237His showed severe phenotypes similar to those with null variants,
while some with p.Ile43Phe or p.Pro387Leu variants were ambulatory and verbal.
Patients 20 and 21, both harboring homozygous p.Ile43Phe variants, started to walk at
the age of 3 and 2.5 years, respectively, and speak short sentences at the age of 4 and 3
years, respectively. (Figure 1b, Supplementary Videos e and f). Videos of patients’
activity are available for Patients 2, 13–15, 20, 21, 26, 30, 36, and 37 (Supplementary
Materials).

Levodopa (L-DOPA) treatment was either ineffective or had an adverse effect,
whereas dopamine receptor agonist treatment mildly improved symptoms in 81%
(17/21) of the patients (Table 1, Supplementary Table 2). Improvement was observed
in alertness, parkinsonism, dystonia, and oculogyric crisis, although it varied with each
patient. The dopamine agonist pramipexole at 0.01–0.02 mg/kg/day divided into 1-3
doses was initiated; if a patient tolerated this dose without adverse effects, it was
The increased dose of pramipexole caused agitation or nausea in a few cases.

**Mapping of disease-causing SLC18A2 missense variants on the VMAT2 structural models**

The structure of human VMAT2 has not been experimentally determined. However, homology models of the rat protein (rVMAT2) have been constructed using related transporter structures as templates, to represent three conformational states in the transport cycle (cytoplasm-facing, occluded, and lumen-facing conformations). These models agree well with a model created by an artificial intelligence-based structure prediction program, AlphaFold2, in terms of distribution of transmembrane helices.

We mapped the positions of missense variants on the structural models of rVMAT2. The models contain two domains (the N- and C-domains), each consisting of six transmembrane helices, and their relative orientation changes between the cytoplasm- and lumen-facing conformations (**Figure 2**). Proline and glycine residues—generally known as “helix breakers”—occur at multiple transmembrane helices and are assumed to facilitate the conformational distortion or flexibility of
VMAT2; this is essential for proton-coupled conformational changes in the transport cycle.\textsuperscript{32} Notably, among the nine variants reported in this study, four affect prolines (p.Pro42Leu, p.Pro161Arg, p.Pro237His, and p.Pro387Leu) and one affects glycine (p.Gly436Ser) in the transmembrane helices. These variants could perturb the conformational dynamics and subsequently, the transport activity of VMAT2.

Specifically, rVMAT2 carrying the p.Pro42Leu substitution (equivalent to human VMAT2 p.Pro42Leu) abolishes serotonin transport activity.\textsuperscript{33} In the cytoplasm-facing conformation, Pro42 and the adjacent Ile43 are located at the bottom of the central cavity, which accommodates the monoamine substrates (Figure 2b). Pro42 and Ile43 are positioned at the bend of transmembrane helix 1 (TM1) in the cytoplasm-facing conformation (Figure 2d); TM1 is stretched in the lumen-facing conformation (Figure 2c and e). Therefore, the p.Pro42Leu and p.Ile43Phe substitutions may affect this conformational transition of TM1, leading to decreased monoamine transport activity.

Similar to Pro42, Ala310 of rVMAT2 (equivalent to human VMAT Ala309) faces the central cavity in the cytoplasm-facing conformation (Figure 2d). It is located on TM7 and is one turn away from Glu313, an essential residue for monoamine transport activity. Glu313 directly binds to the monoamines and/or protons; therefore,
the human p.Ala309Val substitution may affect the transportation. In rVMAT2, Tyr419
(equivalent to human Tyr418) has been proposed to constitute a part of the cytoplasmic
gate. The rVMAT2 variants carrying p.Tyr419Ser and p.Tyr419Ala, but not
p.Tyr419Phe, lack serotonin transport activities.\textsuperscript{31} Therefore, a human VMAT2 variant
carrying p.Tyr418Cys, without an aromatic ring, may exhibit impaired transport
activity.

\textit{Caenorhabditis elegans} model mimicking loss-of-function abnormalities in
\textit{SLC18A2}

To create a \textit{C. elegans} disease model we deleted \textit{cat-1}, the worm homolog of \textit{SLC18A2}.
The mutant showed increased curvature of the head and neck and slow movement, and
was generally more stationary than the wild type reference strain N2 (Supplementary
\textbf{Figure 2a–d}). Upon stimulation with an aversive stimulus (pulses of blue light),
\textit{cat-1}(\textit{syb4974}) demonstrated a hypersensitive, but short lived, photophobic escape
response (Supplementary Figure 2f–i). We noted that the \textit{cat-1}(\textit{syb4974}) phenotype
closely resembled that of another \textit{C. elegans} model of hypotonia, \textit{nca-2}(\textit{syb1612}),
which has a LoF variant in the sodium cation leak channel (unpublished data).
Therefore, \textit{C.elegans} exhibits a conserved multi-dimensional behavioral phenotype
based on the variants in different genes; the symptoms overlap with those in humans with the corresponding variant. The observed behavioral phenotypes are noticeable in short recordings with reasonable throughput, making them an appropriate readout for drug repurposing screens similar to others that have been successful in recording *C. elegans* using whole-organism phenotypes.

**Effects of monoamine on the *C. elegans* model**

Consistent with previous reports on the effects of VMAT variants on monoamine-dependent behaviors in *C. elegans*, the *cat-1(syb4974)* mutant exhibited a decreased pharyngeal pumping rate when foraging on a bacterial lawn (Supplementary Figure 2e). Based on previous studies on the treatment of monoamine disturbances in humans, we exposed the *cat-1(syb4974)* worms to carbidopa, dopamine, levodopa, or pramipexole for 4 h. No significant changes were observed in the phenotypes of any *cat-1(syb4974)* worms, at any of the concentrations (0.1–500 µM), compared with that of the control worms (exposed to the solvent only) (Supplementary Figure 3). The lack of significant modulation by the compounds that we tested may be due to a lack of drug accumulation in the worms and, therefore, longer treatments at higher concentrations should be considered in future studies.
Herein, we described the clinical manifestations observed in 42 individuals from 26 families of different ethnicities with 17 novel and 2 previously reported SLC18A2 variants. Among the affected individuals in the current and previous studies, p.Pro237His is the most common disease-causing variant. This variant is found relatively frequently across multiple variant databases and could be an ancient founder variant. The high mortality rate indicates the poor prognosis of this genetic disorder. Notably, 9 of the 17 patients with p.Pro237His (median age: 5.0 [0.5–14] years) died, whereas all 14 patients with p.Pro387Leu (median age: 11.0 [3–18] years) survived. In addition, although the affected individuals with homozygous SLC18A2 variants were often in bed-bound states, those with p.Ile43Phe and p.Pro387Leu were able to walk, indicating a mild clinical phenotype. In contrast, the affected individuals with p.Pro237His exhibited a severe phenotype and poor prognosis, similar to those of individuals with truncating variants. Consistent with this observation, Jacobsen et al. reported that patients with p.Pro237His showed a more severe phenotype than the individuals with p.Pro387Leu. These observations indicate a phenotype–genotype correlation in SLC18A2-related disorder.
Typical patients with SCL18A2 variants showed global developmental delay, hypotonia, dystonia, parkinsonism, and autonomic nervous system involvement (e.g., temperature dysregulation/sweating, hypersalivation, gastrointestinal dysmotility, and oculogyric dysmotility). Autonomic dysregulation in combination with extrapyramidal movements, in the presence of structurally normal basal ganglia, is an important clinical feature to distinguish cerebral palsy from this neurotransmitter disease. Other neurotransmitter disorders, such as SLC6A3-related DATS and aromatic l-amino acid decarboxylase (AADC) deficiency (MIM# 608643), are possible differential diagnoses, and CSF neurotransmitter analyses are essential diagnostic tests. Typically, SLC6A3-related DATS have a high HVA with a normal 5-HIAA ratio (CSF HVA:5-HIAA ratio >5), whereas AADC deficiency has low levels of both HVA and 5-HIAA. In contrast, both HVA and 5-HIAA levels in the CSF were within the normal limits in this SLC18-related disorder, which is a key finding differentiating other neurotransmitter disorders. These observations are consistent with previous reports.

Both p.Pro237His and p.Pro387Leu variants affect proline residues located on transmembrane helices (Figure 2a, b), and patients with p.Pro237His have severe phenotypes. In the cytoplasm- and lumen-facing conformation of rVMAT2 (Figure 2c and e), Pro238 (equivalent to human VMAT2 Pro237) is located at the interface
between the N- and C-domains, presumably forming a part of the “hinge” region in the conformation of the two domains. Therefore, the Pro237His substitution in human VMAT2 could also affect the conformations of the two domains during the transport cycle. Alternatively, the Pro237His variant could be energetically unstable because of the relatively hydrophilic Pro237His side-chain embedded in the lipid bilayer.

VMAT2 is well established as a therapeutic target.\textsuperscript{29,37} Notably, L-DOPA is either ineffective or worsens the symptoms in patients, whereas dopamine receptor agonists improve the symptoms. The treatment efficacy was difficult to assess due to the short treatment periods; however, a mild symptom improvement was observed in 81\% of the patients treated with a dopamine receptor. This finding is inconsistent with the earliest report describing a dramatic therapeutic effect of a dopamine receptor agonist in a large family of patients with p.Pro387Leu variants.\textsuperscript{7} p.Pro387Leu causes a relatively mild phenotype, and drug responses should be cautiously evaluated alongside phenotype–genotype correlation.\textsuperscript{9} Patients 20 and 21 from Family 11 (with p.Ile43Phe) were able to walk and speak, even without treatment; therefore, certain genetic variants may have a higher effect on illness severity than the treatment itself.

A \textit{C. elegans} model was constructed to mimic the affected individuals with LoF variants; it exhibited slow movement, a hypersensitive but short-lived photophobic
escape response, and a decreased pharyngeal pumping rate. Therefore, the *SLC18A2* LoF could be harmful, at least in worms and humans. We did not observe any rescue of *cat-1* mutant phenotypes in response to dopamine or dopamine agonists at the tested concentrations.

The solute carrier (SLC) protein family is a superfamily of transmembrane transporters with over 400 members that are involved in the exchange of amino acids, nutrients, ions, metals, neurotransmitters, and metabolites across various biological membranes. To date, 287 SLC genes have been found in the brain, and mutations or dysfunctions of over 70 SLC-encoding genes have been shown to be associated with a variety of human brain disorders, such as severe developmental delay or epileptic encephalopathies.

In summary, in combination with previously published data, we identified the genetic and clinical features of 42 newly affected individuals with homozygous *SLC18A2* variants. We elucidated the clinical synopsis of the trait together with functional study results. These findings may facilitate the establishment of genotype–phenotype correlations for *SLC18A2*-associated parkinsonism-dystonia syndrome. This allelic series study provides initial insights into *SLC18A2*-related disorders with both diagnostic and therapeutic implications.
FIGURE LEGENDS

Figure 1. (a) Familial pedigrees of 26 families consisting of 42 affected individuals with homozygous solute carrier family 18 member A2 gene (SLC18A2) variants. (b) Clinical photographs of patients 2, 4–6, 13–16, 19–24, 27, 28, 30, 31, 34, 40, and 42.

Figure 2. Protein structures in two (a) and three (b–d) dimensions. The positions of the disease-causing variants are mapped onto the homology models of rat vesicular monoamine transporter type 2 (rVMAT2). The N- and C- domains are colored green and cyan, respectively. The rat residues and corresponding human disease-causing variants (in parentheses) are indicated. The central cavity, which accommodates substrates in the cytoplasm-facing conformation is indicated using a red dotted line. (b, c). Cytoplasm-facing (b) and lumen-facing (c) conformations. Left, side view. Right, top view from the cytoplasm. (d, e) The structures of transmembrane helix 1 (TM1) and TM7 in the cytoplasm-facing (d) and lumen-facing (e) conformations.

WEB RESOURCES used for this study
AlphaFold Protein Structure Database: https://alphafold.ebi.ac.uk
Combined Annotation Dependent Depletion (CADD):
https://cadd.gs.washington.edu/snv


Genome Aggregation Database (gnomAD): http://gnomad.broadinstitute.org/

Human Gene Mutation Database (HGMD®) professional 2022.2:
http://portal.biobase-international.com/hgmd

MutationTaster: http://www.mutationtaster.org/

Online Mendelian Inheritance in Man (OMIM®): https://www.omim.org/

PolyPhen-2: http://genetics.bwh.harvard.edu/pph2/

Protein Model DataBase: http://srv00.recas.ba.infn.it/PMDB/

Sorting Intolerant From Tolerant (SIFT): http://sift.jcvi.org

SLC TABLES: http://slc.bioparadigms.org/

Tierpsy: https://tierpsy.com/code

UCSC Genome Browser: http://genome.ucsc.edu/

DATA AVAILABILITY

The datasets of this study are not publicly available due to concerns regarding patients’ anonymity. We will supply de-identified data upon request.
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Author Contributions: K.Sai. and R.M. conceived and designed the study, interpreted the data and wrote the manuscript. T.S. and K.O. conducted protein structure analysis.

ETHICS DECLARATION: Written informed consent for publication was obtained from the parents of each patient. Ethics approval for this study was obtained from the Institutional Review Board of Yokohama City University School of Medicine, Baylor College of Medicine, University College London, the Norwegian South-Eastern Regional Ethics Committee, and each genetic analysis center.

REFERENCES


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<table>
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<tr>
<th>Demographic</th>
<th>Missense Variants</th>
<th>Truncating and splice site variants</th>
<th>Total</th>
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<td></td>
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<td>Recurrent variant p.Pro387Leu</td>
<td>Other missense variants</td>
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<tr>
<td>Number of patients</td>
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<td>n = 14</td>
<td>n = 11</td>
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<td>Gender (Male/Female)</td>
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<td>Mortality (Living status)</td>
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<td>Age range (median)</td>
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<td>11.0 years [3–18]</td>
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Table 1. Summary of the clinical features of all patients (current and previously reported) with disease-causing biallelic SLC18A2 variants

*A patient with compound heterozygous variants (c.[895G>C];[835_836delAG] p.[Gly299Arg];[Gln280Glufs*58]) (PMID: 31618753) was excluded from Table 1 because of a lack of detailed clinical information.

LoF, loss-of-function; GDD, Global developmental delay; ID, Intellectual disability; NG, nasogastric; WM, white matter; L-DOPA, levodopa
A global cohort identified 42 patients from 27 families with brain monoamine vesicular transport disease arising from homozygous SLC18A2 variants.