Current knowledge of SLC6A1-related neurodevelopmental disorders

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Advances in gene discovery have identified genetic variants in the solute carrier family 6 member 1 gene as a monogenic cause of neurodevelopmental disorders, including epilepsy with myoclonic atonic seizures, autism spectrum disorder and intellectual disability. The solute carrier family 6 member 1 gene encodes for the GABA transporter protein type 1, which is responsible for the reuptake of the neurotransmitter GABA, the primary inhibitory neurotransmitter in the central nervous system, from the extracellular space. GABAergic inhibition is essential to counterbalance neuronal excitation, and when significantly disrupted, it negatively impacts brain development leading to developmental differences and seizures. Aggregation of patient variants and observed clinical manifestations expands understanding of the genotypic and phenotypic spectrum of this disorder. Here, we assess genetic and phenotypic features in 116 individuals with solute carrier family 6 member 1 variants, the vast majority of which are likely to lead to GABA transporter protein type 1 loss-of-function. The knowledge acquired will guide therapeutic decisions and the development of targeted therapies that selectively enhance transporter function and may improve symptoms. We analysed the longitudinal and cell type-specific expression of solute carrier family 6 member 1 in humans and localization of patient and control missense variants in a novel GABA transporter protein type 1 protein structure model. In this update, we discuss the progress made in understanding and treating solute carrier family 6 member 1-related disorders thus far, through the concerted efforts of clinicians, scientists and family support groups.

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Abbreviations: GAT1 = GABA transporter protein type 1; SLC6A1 = solute carrier family 6 member 1.
Introduction

Solute carrier family member 6 (SLC6A1)-related disorders are emerging as a common cause of developmental and epileptic encephalopathies, since initial descriptions in 2015 (Carvill et al., 2015). SLC6A1 encodes the GABA transporter protein type 1 (GAT1), which is responsible for the reuptake of GABA into presynaptic neurons and glia (Brøer and Gether, 2012). Disruption of SLC6A1 is a prominent cause of neurodevelopmental disorders, including autism spectrum disorder, intellectual disability and seizures of varying types and severity. In the current three largest genomic screens of individuals with epilepsy (8565, 9170 and 9769 patients, respectively), SLC6A1 was listed among the top 10–20 genes with the highest number of pathogenic variants (Lindy et al., 2018; Epi25 Collaborative, 2019, p. 25; Truty et al., 2019). In the most extensive autism sequencing study to date (N = 11 986), SLC6A1 was among the top 10 genes, with the most significant variant enrichment in autism patients compared to 23 598 controls (Satterstrom et al., 2019). Recently, exome sequencing of individuals with schizophrenia found rare de novo missense variants in SLC6A1 to be associated with schizophrenia in three patients (Rees et al., 2020), extending the phenotype spectrum beyond epilepsy. Overall, the incidence of SLC6A1-related disorders is estimated to be 2.65 (90%CI: 2.38–2.86) per 100 000 births (López-Rivera et al., 2020).

Following the second SLC6A1 Symposium organized by the SLC6A1 Connect Foundation (https://slc6a1connect.org, 1 October 2020, date last accessed) that convened academic scientists, physicians and family advocacy organizations, we summarize the current state of research and future directions. We collected and curated the largest dataset of SLC6A1 variants to date with accompanying clinical phenotyping (N = 116). Our study represents a significant step forward to define the clinical and genotypic spectrum of SLC6A1-related disorders and ultimately will help to guide clinical management.

The SLC6A1 gene

The SLC6A1 gene is located on chromosome 3 (Genome Research Consortia human assembly version 38 genomic coordinates: 3:10 992 733–11 039 248) and contains 15 exons. The encoded GAT1 protein has 12 transmembrane domains that form a single chain transporter (Fig. 1A). The primary function of GABA transporters is to lower the concentration of GABA in the extracellular space (Scimemi, 2014) (Fig. 1B). There are six major splice isoforms of human GAT1 that differ by alternative use of exons three to five. The transcript ENST00000287766 is the longest isoform of SLC6A1 and is considered canonical (Hunt et al., 2018); accordingly, most genetic variants are mapped into its sequence. The exact topology of GAT1 remains unclear due to the lack of a mammalian crystal structure. Still, homology models based on a 20–25% sequence identity to GAT1 (Yamashita et al., 2005) have allowed the identification of essential residues for substrate and sodium binding in transmembrane domains one, three, six and eight, which are necessary for the conformational transitions during the transport process (Fig. 1A and B). The SLC6A1 gene belongs to a gene family of 20 paralogues. The proteins encoded by 13 of these genes exhibit above 80% sequence identity, and six of them can transport GABA with different degrees of substrate specificity. These paralogues have been associated with a variety of neurodevelopmental disorders (Brøer and Gether, 2012). Upon linear protein sequence alignment of the 13 most paralogue-conserved gene family members, disease-associated variants reported in different family members cluster significantly together in two amino acid regions in comparison to variants from the general population. These regions are known as pathogenic variant enriched regions and any missense variant found within is 106 times more likely to be classified as pathogenic than benign (Fig. 1C) (Pérez-Palma et al., 2020). However, as in the case of many other genetic etiologies linked to neurodevelopmental disorders, disease-causing variants in SLC6A1 among affected individuals are broadly distributed along its sequence (Johannesen et al., 2018).
Figure 1  SLCA6A1 main features. (A) Schematic representation of the 2D structure of GAT1. (B) Diagram of GAT1 GABA transport function. (C) Paralogue conservation of the SLC6A1 gene family members. Higher values denote the degree of conservation. The pathogenic enriched regions are shown in red bars. (D) Cell type-specific expression of SLC6A1 and other frequently mutated epilepsy and neurodevelopmental disorder-associated genes (http://celltypes.brain-map.org/maseq/human). (E) Dynamic gene expression of SLC6A1 along with entire development and adulthood in the cerebellar cortex (CBC), mediodorsal nucleus of the thalamus (MD), striatum (STR), amygdala (AMY), hippocampus (HIP) and 11 areas of the neocortex (NCX) (http://hbatlas.org).
GABA receptors is prolonged, whereas the frequency, membrane potential or a shunt for membrane resistance. currents, GAT1 activation can create local changes in membrane potential or a shunt for membrane resistance. In GAT1 deficient mice, tonic inhibition is increased and the decay time of evoked phasic currents mediated by GABA_A receptors is prolonged, whereas the frequency, amplitude and kinetics of spontaneous GABA_A postsynaptic currents is not changed (Jensen et al., 2003; Chiu et al., 2005; Bragina et al., 2008; Cope et al., 2009).

Interestingly, other works on GAT1-deficient mice show that the frequency of miniature inhibitory postsynaptic currents is decreased, an effect that is associated with increased expression of enzymes that contributes to GABA synthesis in presynaptic terminals of inhibitory neurons (i.e. GAD65/67, periaqueductal gray matter) (Bragina et al., 2008; Conti et al., 2011). Other works in the striatum indicate that GAT1 shape GABAergic transmission through pre- and post-synaptic mechanisms. Together, these findings highlight multiple molecular and cellular functions of GAT1 that demonstrate the complex etiology leading to clinical symptoms of patients with SLC6A1-related disorders. Given that GABA homeostasis is critical for brain development, it is plausible that early intervention strategies will be essential for the well-being of the patients.

### SLC6A1-related disorders

In 2015, a 3p microdeletion involving only SLC6A1 and SLC6A11 was described in a patient with Doose Syndrome, a developmental epileptic encephalopathy associated with intellectual disability and early-onset epilepsy with myoclonic atomic seizures (previously myoclonic atomic epilepsy). Furthermore, likely pathogenic variants in SLC6A1 were identified in 6/160 (4%) individuals with a previously undiagnosed early-onset epilepsy with myoclonic atomic seizures (Carvill et al., 2015) and additional studies identified individual patients with autism spectrum disorder and developmental epileptic encephalopathy carrying variants in SLC6A1 (Rauch et al., 2012; Sanders et al., 2012). In the first case series, 34 patients with variants in SLC6A1 were clinically described in 2018 (Johannesen et al., 2018). Notably, nearly all genetic variants reported arose de novo and not present in the general population (Lek et al., 2016).

Here we present the largest collection of SLC6A1 patients, including genetic and clinical features (Supplementary Table 1). Our cohort includes 116 patients from three sources: previously reported individuals (38.6%), individual referrals to the SLC6A1 Connect Foundation (15.5%), and the Epi25 Collaborative for Large-Scale Whole Genome Sequencing in Epilepsy Collaborative Database (25.8%) (Epi25 Collaborative, 2019). Clinical and genetic data are presented in Supplementary Table 1. Data from the Epi25 Collaborative for Large-Scale Whole Genome Sequencing in Epilepsy Collaborative database were limited to genotype and International League Against Epilepsy categorization only. We describe 85 unique SLC6A1 variants. Most of patients variants observed arose de novo (40/49, 81.63%). Regarding variant type, we observed 88 missense variants, 15 protein truncating variants) that lead to a complete loss of function, seven variants in splice sites, three large deletions Copy Number Variants, two small insertions and deletions and one synonymous variant (Supplementary Table 1). In our cohort, epilepsy (92/101, 91.1%), developmental delay, and cognitive impairment (46/56, 82.1%) and autistic traits (20/92, 22.8%) were the most common clinical features (Fig. 2A). We found a similar number of males and females (55.1% female), and the mean age of seizure onset was 2.5 years (standard deviation 1.58 years). Developmental data concerning seizure-onset was available on 43 before seizure-onset and 35 after seizure-onset. Developmental delay was present in 26/43 (60.4%) before seizure-onset. After the onset of seizures, nearly all subjects demonstrate developmental delay (46/55, 83.6%), most in the mild to moderate range (35/55, 63.6%) (Fig. 2B). The most prevalent epilepsy syndrome was early-onset epilepsy with myoclonic atomic seizures (20/82, 24.3%) followed by genetic generalized epilepsy (19/82, 23.1%) and non-acquired focal epilepsy (8/82, 9.75%) (Fig. 2C). Detailed seizure semiology data was available on 56 of the 92 subjects with epilepsy; most commonly (atypical) absence seizures (38/53, 71.7%), atomic seizures (24/54, 44.44%) and myoclonic seizures (15/54, 27.77%) (Fig. 2D). Generalized epileptiform discharges (36/52, 69.2%) were the most commonly reported EEG abnormality among available data, especially at a frequency of 2–4 Hz (12/52, 23%). Generalized background slowing is reported in 17/52 (32.6%). Recently, an exome-wide trio sequencing study identified de novo missense variants in SLC6A1 to be associated with schizophrenia (Rees et al., 2020). No evidence of epilepsy, intellectual disability, or autism spectrum disorders was reported in the three patients described (Supplementary Table 1).

### Towards a model of SLC6A1 pathophysiology

Missense variants can cause a gain of function or loss of function; however, the expected molecular mechanism by which variants lead to SLC6A1-related disorders is loss of function or haploinsufficiency. This disease-model is supported by in vivo and in vitro experiments in both wild type and GAT1 knockout mice, as well as studies on re-combinant GAT1 proteins from individuals with SLC6A1
variants. However, the mechanisms by which the loss of function lead to clinical manifestations are not well understood. Recently, experimental evidence showed that seven SLC6A1 variants (five missense variants, one in-frame deletion and one nonsense variant) identified in epilepsy patients reduce GABA transport in vitro studies (Mattison et al., 2018). The residual transporter activity was found, ranging from 2% to 27% compared to the wild type.

It has been shown that SLC6A1 variants also cause impaired protein trafficking. Characterization of a missense variant in SLC6A1 (G234S) associated with Lennox–Gastaut syndrome leads to reduced protein expression in both cell surface and total protein levels in heterologous and rat cortical neurons (Cai et al., 2019). The surface protein level of the mutant is about 70% of the wild type, while the GABA uptake of the mutant GAT1 is ~30% of the wild type. This suggests the mutant GAT1 had impaired GABA uptake in addition to impaired protein trafficking (Cai et al., 2019). Similarly, a recent report on SLC6A1 (P361T) associated with epilepsy and autism indicates that the mutant GAT-1 had endoplasmic reticulum retention and enhanced degradation (Wang et al., 2020).

There is no specific animal model of SLC6A1-related disorders. Heterozygous (Het) GAT1 knockout mice are phenotypically normal despite having diminished GABA reuptake capacity (Chiu et al., 2005; Cope et al., 2009). The homozygous GAT1 knockout animals exhibit a constant tremor, abnormal gait, reduced strength, absence seizures and mobility, as well as anxious behaviors (Chiu et al., 2005; Cope et al., 2009). This model partially recapitulates features of the human disease, including mobility and cognitive impairment (Johannesen et al., 2018). Ex vivo hippocampal and thalamic recordings show that GAT1 homozygous knockout mice have an increase in
GABA$_{	ext{A}}$R tonic inhibition (Jensen et al., 2003; Cope et al., 2009), which is known to contribute to generalized spike-wave discharge typical of absence seizure (Crunelli V et al., 2020).

**Prediction of GAT1 structure and evaluation of missense variants**

Truncating variants are very likely to lead to loss-of-function due to lower levels of GAT1 and subsequent haploinsufficiency. However, the molecular consequence of missense variants can range from benign to damaging, mostly determined by the effect of the variants on protein function. We explored the pathogenic variants’ positions in the GAT1 sequence in the context of the functionally critical sites and domains (Fig. 3A). To further investigate the benign variants from the general population and pathogenic variants from patients in 3D space, we generated the structure of GAT1 using the RaptorX server [Fig. 3B, based on template structure: 4XPT (Wang et al., 2015)]. We then mapped the variants’ positions on the structure (Fig. 3C and D). Differential spatial segregation of variants is notable upon 3D mapping. The majority of the patient variants are located in the helical-transmembrane segments (32 out of 88, 36.4%) and inter-helical hinges (42 out of 88, 47.8%) (Fig. 3D, left) while genome aggregation consortia variants cluster in the cytoplasmic domain (38 out of 121, 31.4%, Fig. 3C). We observed ten patient variants also present in the general population (genome aggregation consortia) (Supplementary Table 1). The pathogenicity of these variants can be challenged according to the ACMG guidelines (Richards et al., 2015). We further highlighted the amino acid positions harboring recurrent patient variants and those also found in genome aggregation consortia (Fig. 3D, right). Out of 13 recurrent variant positions, six positions form a cluster near the extracellular (top) part of the structure. All three schizophrenia-related variants’ positions are located on the outer surface of the structure, mutated residues more than 70% exposed to solvent as measured by the residue’s accessible surface area.

**Existing treatment**

Available clinical data for individuals with disease-causing variants in SLC6A1 is limited, and most patients require an interdisciplinary team, including neurologists, developmental pediatricians, genetic counsellors and speech and occupational therapists for comprehensive management (Kuo et al., 2016; Katkin et al., 2017). There is insufficient data available to guide pharmacotherapy in SLC6A1-related disorders. Thus treatment is guided by existing strategies for the specific clinical epilepsy syndromes, rather than underlying genetic etiology, using broad-spectrum anti-seizure medications, including valproic acid, lamotrigine or benzodiazepines. In a prior study, 20 of 31 patients became seizure-free with anti-seizure medication, and valproic acid was the most effective drug. Lamotrigine and ethosuximide also showed success (Johannesen et al., 2018). Importantly, there was not a correlation between seizure control and cognitive outcome.

**Future treatment**

There is a clear unmet medical need for improved treatment options for SLC6A1-related disorder. GAT1 is a potential candidate for therapeutic development based on the following observations: (i) it has a known biological function based on *in vitro* and *in vivo* studies (Jensen et al., 2003; Scimemi, 2014), (ii) there are known antiepileptic drugs such as valproic acid, tiagabine and vigabatrin that can modulate GABA concentrations (Schousboe et al., 2014) and (iii) the transporters’ relatively small size (599 aa) makes SLC6A1 a plausible candidate for viral-mediated gene therapy using adeno-associated virus vectors (Chamberlain et al., 2016). Alternatively, antisense oligonucleotides therapy can be used to specifically increase productive SLC6A1 mRNA and consequently restore levels of GAT1 protein. Antisense oligonucleotides strategies targeting non-productive splicing events have shown promising results in animal models of other haploinsufficiency neurodevelopmental disorders like Dravet syndrome (Han et al., 2020; Lim et al., 2020).

Restoration of the GAT1 transporter function may provide therapeutic benefit, but two challenges arise. First, is the need to understand the regulation of GAT1 expression and activity in a developmental context, and second, the reversibility of symptoms is unknown. Patient-derived induced pluripotent stem cell experiments (i.e. *in vitro* neuronal cultures and organoids) allow researchers to probe for the functional effects of SLC6A1 variants on neuronal excitability, developmental progression and network behavior. Importantly, this can be done in human neurons, under physiological expression levels of the transporter and under each patient’s unique genetic background. Transgenic rodent models provide the opportunity to assay neurons in the context of the brain to capture non-autonomous, circuit-level and behavioral effects. Understanding how alterations in GAT1 function affect both developing and mature neuronal integration, as well as network function, is critical to enable effective interventions.

**Towards precision therapeutics**

To develop treatments for patients with SLC6A1-related disorders it is critical to define the full phenotypic
spectrum of the disease. Observational studies are needed to characterize the natural course of the disease and to identify appropriate end-points for use in future interventional trials. This cross-sectional review of available clinical data is a critical first step. Based on the expanded cohort described here, epilepsy and neurodevelopmental disabilities appear to be the core features of SLC6A1-related disorders. However, additional assessments of gait and mobility or movement disorders may also be of interest.

Further progress will require collaboration between clinicians, scientists and family organizations such as the SLC6A1-Connect Foundation (https://slc6a1connect.org). Significant limitations of a retrospective dataset include the lack of uniformity of presented data and risk that the same patient may be presented in multiple de-identified datasets.

Figure 3 GAT1 3D structure model and spatial distribution of patient and general population missense variants in SLC6A1.

There is no experimentally solved protein structure of the eukaryotic GAT1 protein (Fig. 2A). We predicted the GAT1 structure using a homology model from a dopamine transporter (Protein Data Bank ID: 4xp4) (Waterhouse et al., 2018) and a multi-template based serotonin transporter (Protein Data Bank IDs: 4xpt, and 6awn) using RaptorX web server (Wang et al., 2016). The resulting novel model for GAT1 exhibited high quality and confidence (P-value = 3.67e-13). (A) Linear GAT1 protein sequence. The 599-residue long sodium- and chloride-dependent GAT1 consists of 12 helical domains (light purple), 1 extracellular (light green) and 2 cytoplasmic (light orange). (B) Predicted 3D structure, domains (colored as in A), and special binding sites (colored spheres). Numbers (in red) above the reference amino acids in sequence reflect the count of patients carrying a missense variant altering that amino acid. (C) Genome aggregation consortia variants (blue spheres) variant positions mapped on 3D and (D) patient variants (orange spheres). Recurrent variants are highlighted in red (upper-left structure) as well as patient variants also found in the general population (N = 10, bottom-left structure).
datasets. Collaborations between crucial stakeholders will lead to the development of disease-specific tools for collecting and analysing patient data, including greater data consistency, longitudinal phenotyping and standardized evaluation of medication use and response. More extensive population studies may also elucidate relationships between SLC6A1 variants and milder phenotypes. Furthermore, the use of EEG and imaging biomarkers can be explored. EEG may prove to be a valuable biomarker, given the high prevalence of abnormal electrical activity in clinical reports, however little is known about the EEG signature in individuals with SLC6A1-related disorder without seizures. Finally, standardized neuro-psychological assessments to characterize developmental disabilities are needed, including patient-centred outcome measures that are jointly developed with substantial input from patient advocates and family organizations.

A key challenge in the pursuit of precision therapeutics is the identification of eligible individuals. While access to genetic testing is improving, it remains a significant barrier in some countries. Identification of early phenotypic features that should prompt appropriate evaluation will support early diagnosis. Alongside deep phenotyping, functional analysis of variants will be necessary to assess genotype-phenotype correlations. Finally, the implementation of high-throughput analysis of the functional impact of variants on channel function would accelerate drug development considerably.

Conclusion

SLC6A1-related disorders are neurodevelopmental disorders caused by aberrant GABA neurotransmission secondary to impaired functioning of GAT1. Based on a review of 116 individuals with SLC6A1-related disorder, developmental delay, epilepsy, autism and motor dysfunction, including stereotypies and ataxia, are the most common clinical features. Data from the literature and our analysis on GAT1 structure support loss-of-function as the primary disease-associated molecular pathology. A comprehensive translational research programme needs to be developed (i) to better understand the underlying pathophysiology, (ii) to develop targeted therapies for SLC6A1-related disorder and (iii) to define the full clinical spectrum of the disease.

Supplementary material

Supplementary material is available at Brain Communications online.

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Competing interests

The authors report no competing interests.

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