REPORT

# Dysfunction of the Cerebral Glucose Transporter SLC45A1 in Individuals with Intellectual Disability and Epilepsy

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Glucose transport across the blood brain barrier and into neural cells is critical for normal cerebral physiologic function. Dysfunction of the cerebral glucose transporter GLUT1 (encoded by *SLC2A1*) is known to result in epilepsy, intellectual disability (ID), and movement disorder. Using whole-exome sequencing, we identified rare homozygous missense variants (c.526C>T [p.Arg176Trp] and c.629C>T [p.Ala210Val]) in *SLC45A1*, encoding another cerebral glucose transporter, in two consanguineous multiplex families with moderate to severe ID, epilepsy, and variable neuropsychiatric features. The variants segregate with the phenotype in these families, affect well-conserved amino acids, and are predicted to be damaging by in silico programs. Intracellular glucose transport activity of the p.Arg176Trp and p.Ala210Val SLC45A1 variants, measured in transfected COS-7 cells, was approximately 50% ($p = 0.013$) and 33% ($p = 0.008$) lower, respectively, than that of intact SLC45A1. These results indicate that residues at positions 176 and 210 are critical for the glucose transport activity of SLC45A1. All together, our data strongly suggest that recessive mutations in *SLC45A1* cause ID and epilepsy. SLC45A1 thus represents the second cerebral glucose transporter, in addition to GLUT1, to be involved in neurodevelopmental disability. Identification of additional individuals with mutations in *SLC45A1* will allow better definition of the associated phenotypic spectrum and the exploration of potential targeted treatment options.

Glucose is the major energy fuel of the central nervous system. However, its availability is restricted given the selective permeability of the blood brain barrier (BBB) and the relative lack of carbohydrate stores in the brain. Thus, glucose transport across the BBB and into neural cells is critical for cerebral physiologic function and energy metabolism (for a review, see Chen et al.[1]). GLUT1 is the key regulator of glucose across the BBB.[1] The importance of this process is illustrated by the observation that dominant mutations in *SLC2A1* (OMIM: 138140), which encodes GLUT1, cause epilepsy with variable degrees of intellectual disability (ID) and/or movement disorder.[2] Once glucose enters the brain's extracellular space, it is taken up by different cerebral cells through various glucose transporters, which are expressed in developmentally and cell-type-specific manners. Whether disruption of these neural glucose transporters also causes neurodevelopment disorders remains unknown.

We report on four affected children from two unrelated consanguineous families with moderate to severe ID associated with epilepsy and variable neuropsychiatric features. Using whole-exome sequencing, we identified homozygous missense variants in *SLC45A1* (OMIM: 605763), which codes for a neuronal glucose transporter that is predominantly expressed in the developing and adult brain.[3] Functional studies indicate that the identified variants reduce the activity of the protein, thus implicating SLC45A1 dysfunction in the etiology of ID and epilepsy. SLC45A1 could thus represent the second cerebral glucose transporter, along with GLUT1, to be involved in neurodevelopmental disability.

Individuals A.II-2 and A.II-3 from family A are Palestinian sisters whose parents are first-degree cousins (Figure 1A). Individual A.II-2 is currently 22 years old. She was born after an uncomplicated pregnancy and delivery. Her birth weight was 3,750 g (75$^{th}$ percentile), and her length was 50 cm (50$^{th}$ percentile). She had a patent ductus arteriosus (PDA) repair during infancy. Gross motor development was normal, but language development was delayed. She has moderate ID and has always attended special schools. She can read and write simple sentences. She is able to take the bus independently and hold a simple job. She developed focal seizures with impaired awareness at the age of 4.5 years. These seizures were characterized by epigastric discomfort followed by staring and eye blinking lasting less than 1 min. Electroencephalography (EEG) demonstrated focal epileptic activity mainly over the left fronto-temporal region but also at times over the right fronto-temporal region. Background activity was normal. Her seizures were well controlled on a combination of carbamazepine and clobazam. At the age of 9 years, she developed a 45 min secondarily generalized tonic-clonic status epilepticus in the context of fever, 3 months after her antiepileptic medications were discontinued. She was restarted

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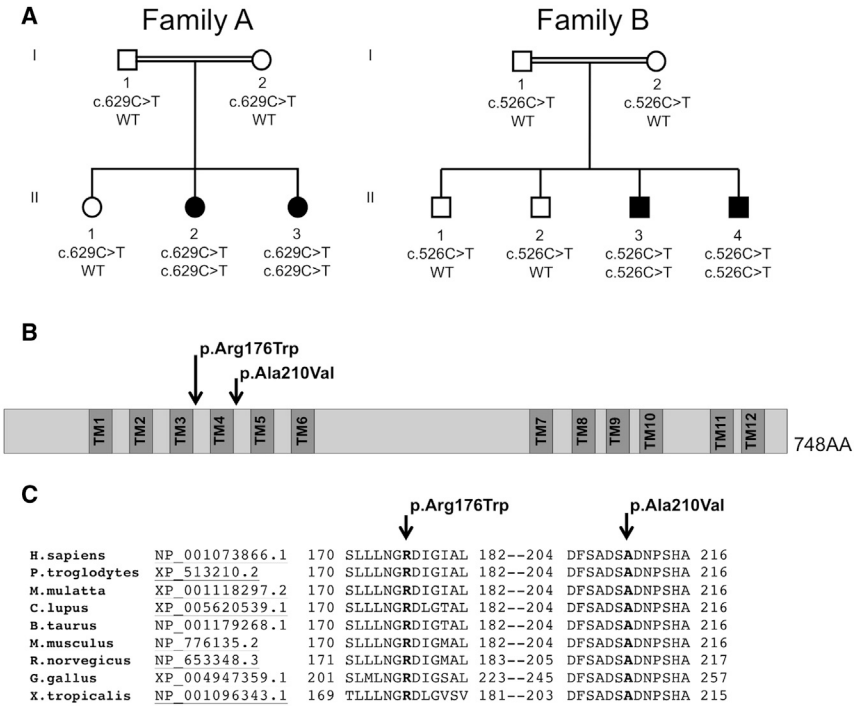


**Figure 1. Recessive *SLC45A1* Mutations in Individuals with ID and Epilepsy**
(A) Segregation of *SLC45A1* mutations within the affected families.
(B) Schematic diagram showing the various domains of SLC45A1 and the relative position of the p.Arg176Trp and p.Ala210Val substitutions. Domain locations are based on UniProt (see Web Resources).
(C) Alignment of amino acid residues near the positions p.Arg176 and p.Ala210 of human SLC45A1 (*H. sapiens*; GenBank: NP_001073866.1) and its orthologs. Amino acid alignments were generated with NCBI HomoloGene.

on clobazam and carbamazepine, which she continues to take, and hasn't had any seizure recurrence. She is very anxious and has some obsessive-compulsive tendencies, such as compulsively cleaning or eating, although this behavior has not significantly interfered with her daily activities. On her last examination at the age of 19 years, her weight was 72.5 kg (80$^{th}$ percentile), her height was 157 cm (20$^{th}$ percentile), and head circumference was 54 cm (40$^{th}$ percentile). She has downslanting eyes, a smooth philtrum, and a thin upper lip. Her neurological examination was unremarkable except for slowness during finger-to-nose testing and rapid-alternating-movement testing. Brain MRI and karyotype (450 bands) were normal.

Subject A.II-3 is currently 20 years old. She was born after an uneventful pregnancy and delivery. She attained early milestones appropriately but had significant difficulties at school and was diagnosed with moderate ID. She is able to walk, speak in sentences, and understand complex commands. She can read and write and has always attended special classes for children with ID. At the age of 11 years, she developed focal seizures accompanied by impaired awareness consisting of staring episodes, epigastric discomfort, fumbling with her clothes, chewing, and right facial twitches lasting 30–60 s. She has had occasional secondarily generalized seizures. EEG video telemetry during ictal events captured focal spike-and-wave epileptic activity over the right fronto-temporal region. Seizures originating from the left temporal lobe were also recorded. Seizures were initially poorly controlled despite the trial of multiple anticonvulsants. She has had no seizure recurrence since the age of 17 years on a combination of carbamazepine and clobazam, which she continues to take. This individual has significant neuropsychiatric and behavioral issues. She has important difficulties with social integration, obsessive-compulsive disorder with repetitive behaviors (e.g., repeatedly saying the same thing, repeating the same action, and echolalia), and tangential speech that seems to worsen with age. She was treated by a psychiatrist, who diagnosed her with autism spectrum disorder. She has had multiple neuropsychological assessments (at the ages of 11, 12, 16, and 19 years), which have not documented any cognitive decline. She cannot live independently, nor can she hold a simple job. At the age of 16 years, she developed tics such as eye blinking, eye deviation, and throat clearing. She has scoliosis. At the age of 16 years, this individual was started on the modified Atkins diet in an attempt to improve her behavior; she was having focal seizures with impaired awareness every 2–3 months. The diet consisted of a 2:1 ketogenic ratio (grams of fat to combined carbohydrates and protein) and a net carbohydrate total of 10 g per day. It was discontinued after 6 months because she had difficulties with adhering to the diet and showed no clear improvements in either behavior or seizure frequency. On physical examination at the age of 16 years, her weight was 52.5 kg (45$^{th}$ percentile), height was 149 cm (2$^{nd}$ percentile), and head circumference was 53.5 cm (25$^{th}$ percentile). Her facial features (downslanting eyes, a smooth philtrum, and a thin upper lip) are similar to those of her sister. Neurological examination was normal except for bilateral tight heel cords, normal reflexes, and slowness during rapid-alternating-movements and finger-to-nose testing. Brain MRI, chromosomal microarray, serum amino acids, urine organic acids, very-long-chain fatty acids, lactate, blood gas (including $pCO_2$), and ammonia were normal. Amino acids and lactate in the cerebrospinal fluid (CSF) were normal. The CSF glucose was normal (60 mg/dL; age-related cutoff = 48.7 mg/dL[4]), and the ratio of CSF to blood glucose was in the normal range (0.70; age-related cutoff = 0.57[4]; non-fasting blood glucose: 85 mg/dL).

This study was approved by our institutional ethics committee, and informed consent was obtained from each

participant or legal guardian. We performed whole-genome SNP genotyping in the one unaffected and two affected siblings by using the Illumina Human 610 Genotyping BeadChip panel, which interrogates 620,901 SNPs. Given that transmission of the phenotype in this family was consistent with autosomal-recessive inheritance and that the parents were consanguineous, we used PLINK[5] to identify regions that contained >30 consecutive SNPs, extended over >1 Mb, and were homozygous in both affected individuals but not in the unaffected sibling. We then performed whole-exome sequencing in affected individual A.II-3. Blood genomic DNA was captured with the Agilent SureSelect Human All Exon Capture V4 Kit and sequenced (two paired-end 100 bp reads and four exomes per lane) with an Illumina HiSeq 2000 at the McGill University Genome Quebec Innovation Center. Sequence processing, alignment (with a Burrows-Wheeler algorithm), and variant calling were performed according to the Broad Institute Genome Analysis Toolkit (GATK v.4) Best Practices, and variants were annotated with ANNOVAR. The average exome coverage of the target bases was 119×, and 95% of the target bases were covered by at least ten reads. Only the variants whose positions were covered ≥8× and supported by at least three variant reads constituting at least 20% of the total reads for each called position were retained. To identify potentially pathogenic variants, we selected variants affecting coding and splice sites that were present at minor allele frequencies (MAFs) ≤ 0.005 in public databases (e.g., 1000 Genomes and NHLBI Exome Sequencing Project [ESP] Exome Variant Server) and ≤1% in in-house control datasets.

We found no rare homozygous or potentially compound-heterozygous variants in genes associated with ID. We then searched the whole-exome dataset for genes harboring homozygous variants in the previously identified homozygous regions (≥1Mb) shared by the two affected sisters but not by their unaffected sibling. Segregation of these variants within the family was confirmed by Sanger sequencing. Variants that were found to be homozygous in the parents were excluded. The five homozygous variants that remained after our segregation analysis were screened in 95 ethnically matched control individuals. Three variants were excluded because they were observed at high frequencies, which are incompatible with a rare recessive disease. The variant c.153C>A (p.Cys51X) in *MIB2* (OMIM: 611141; GenBank: NM_001170688.1) was identified in 3.2% (3/95) of control individuals, c.2103C>A (p.Asp701Glu) in *MMEL1* (GenBank: NM_033467.3) was found in 3.2% (3/95), and c.2135G>A (p.Cys712Tyr) in *ANLN* (OMIM: 616027; GenBank: NM_018685.4) was found in 2.1% (2/95). The remaining candidate variants, c.629C>T (p.Ala210Val) in *SLC45A1* (OMIM: 605763; GenBank: NM_001080397.1) and c.1225C>T (p.Arg409Trp) in *TNFRSF8* (OMIM: 153243; GenBank: NM_001243.4), were both absent in the 95 ethnically matched control individuals. *TNFRSF8* is a member of the tumor necrosis factor receptor superfamily and is a surface antigen used as a clinical marker for Hodgkin lymphoma and related hematologic malignancies. It has no known role in neurodevelopment and is not highly expressed in the brain (Human Protein Atlas). The c.1225C>T (p. Arg409Trp) variant is rare (MAF = 0.00001660 in the Exome Aggregation Consortium [ExAC] Browser) and predicted to be benign by PolyPhen-2 (score = 0.008) and SIFT (score = 0.39). *SLC45A1* encodes a glucose transporter that is highly prevalent in the brain.[3] The c.629C>T (p.Ala210Val) variant is rare (MAF = 0.0001497 in the ExAC Browser) and predicted to be damaging by PolyPhen-2 (score = 0.999) and SIFT (score = 0.000).

In parallel, we identified two ID-affected brothers from the United Arab Emirates, and their parents are also first-degree cousins (Figure 1A). Individual B.II-3 is currently 8 years old and was born after an unremarkable pregnancy and delivery. His birth weight was 2.8 kg ($10^{th}$ percentile), but length and head circumference were not recorded. His development was globally delayed. He walked at 2.5 years and is now able to run and go up and down stairs. He is nonverbal and only produces sounds and jargon. He does not understand simple instructions. He has good eye contact but does not point to needs, share interests, or seek social interaction. He has no pretend play. He spins and flaps his hands. He has had several assessments for a diagnosis of autism, but formal assessment gave conflicting results. He is able to eat with his fingers but does not use cutlery. He needs assistance in most activities of daily living and is not toilet trained. He had one febrile seizure at the age of 7 months and then one afebrile secondarily generalized seizure at the age of 9 months, after which he was started on levetiracetam. The parents discontinued the anticonvulsant when the child was 12 months old, and he has not had any seizure recurrence. EEG revealed enhanced spike-and-slow-wave activity in the left occipital region during sleep, as well as slowing of the background in the left occipital region in the form of polymorphic delta waves. Photosensitivity and electrical status epilepticus during sleep were absent. This individual has a single left kidney. Hyperparathyroidism was diagnosed at the age of 6 years, and hypercalcemia and hypophosphatemia were noted in the context of routine blood work, although the individual was asymptomatic. Head circumference at the age of 18 months was at the $10^{th}$ percentile. On physical examination at 8 years, his height was 118 cm ($3^{rd}$ percentile), and his weight was 19 kg ($<3^{rd}$ percentile). He has a triangular face, downslanting eyes, arched eyebrows, a smooth philtrum, and thin lips. His neurological examination was unremarkable. Investigations that included brain MRI, chromosomal microarray, fragile X testing, transferrin isoelectric focusing, and echocardiography were normal.

His brother, individual B.II-4, is 11 months old. He was born at term with intrauterine growth retardation. His birth weight was 2.2 kg ($<2^{nd}$ percentile), his length was

47 cm (10th percentile), and his head circumference was 36 cm (75th percentile). He failed to thrive in the first months of life. He was found to have a coarctation of the aorta, which was surgically corrected at the age of 7 weeks. An initial renal ultrasound revealed multiple echogenic foci within the medulla of the left kidney, suggestive of nephrocalcinosis, which resolved on follow-up imaging. At the current age of 11 months, he has global developmental delay. He has a head lag and cannot sit unless supported. He can roll over and reach for objects. He can fix and follow visually. He can smile but does not seem to recognize his mother. He has not had any seizures. On his most recent examination (age 11 months), his weight was 7.5 kg (<3rd percentile), but he was gaining weight very slowly. His height was 72 cm (25th percentile), and head circumference was 45 cm (25th percentile). He had dysmorphic features similar to those of his affected brother. He had a flat, triangular face with a smooth philtrum, thin lips, widely spaced eyes, a depressed nasal bridge, and a facial hemangioma. He was hypotonic and hyporeflexic.

The exome of individual B.II-3 was clinically sequenced with the Roche Nimblegen VCRome v.2.1 exome capture kit and Illumina HiSeq 2000 at the Whole Genome Laboratory at Baylor College of Medicine and analyzed as previously described.[6] No pathogenic or likely pathogenic variants that could explain the disease were reported. Interestingly, we found that B.II-3 bears a homozygous c.526C>T (p. Arg176Trp) variant in *SLC45A1* (GenBank: NM_001080397.1). This variant is very rare (MAF = 0.00001660 in the ExAC Browser) and predicted to be damaging by PolyPhen-2 (score = 0.989) and SIFT (score = 0.000). Segregation of the variant was verified by Sanger sequencing in the family. B.II-4 was also found to be homozygous, whereas his unaffected siblings and parents were heterozygous for this variant (Figure 1A).

*SLC45A1*, also known as *PAST-A* and *DNB5*, is predominantly expressed in the developing and adult brain, including the cortex and cerebellum.[3,7] Several observations suggest that SLC45A1 functions as a glucose transporter. First of all, its primary structure displays classic features of glucose transporters belonging to the major facilitator superfamily, including the presence of 12 putative membrane-spanning helices, a long cytoplasmic loop between TM6 and TM7, and several motifs associated with the transport of sugar.[3] Moreover, transfection studies have established that SLC45A1 is a membrane-associated protein that possesses glucose and galactose (but not sucrose or fructose) transport activity.[3] The uptake of glucose in this assay was pH dependent, such that it increased significantly at low pH value. Finally, the *Drosophila* gene *slc45-1*, a homolog of *SLC45A1* (and an ortholog of *SLC45A2*), also encodes a membrane protein that can transport sugar.[8] Collectively, these observations strongly suggest that SLC45A1 is a $H^+$ and glucose symporter.

Both identified variants, c.526C>T (p. Arg176Trp) and c.629C>T (p.Ala210Val), are located in exon 3 of *SLC45A1* (GenBank: NM_001080397.1). Arg176 is located in the extracellular loop just after the third transmembrane domain, whereas Ala210 is positioned at the beginning of the intracellular loop just after the fourth transmembrane domain (Figure 1B). Arg176 and Ala210, as well as their flanking amino acids, are conserved in mammals, amphibians, and birds (Figure 1C). We sought to biologically validate these variants by examining their effect on the glucose transport activity of SLC45A1. *SLC45A1*-encoding cDNA was inserted into the pFN21A expression vector (Promega). Each missense mutation (c.526C>T and c.629C>T) was introduced to a separate SLC45A1 plasmid with QuikChange Multi Site-Directed Mutagenesis Kits (Agilent Technologies). We sequenced the entire constructs to verify the presence of the mutations and the absence of artifacts. We transfected COS-7 cells with the indicated plasmid by using the cationic lipid-based transfection reagent Lipofectamine 2000 (Thermo Fisher Scientific). The glucose transport assay was performed as described by Shimokawa et al.[3] In brief, 64 hr after transfection, cells were washed with KRPH buffer (20 mM HEPES [pH 7.4], 5 mM $Na_2HPO_4$, 1.25 mM $MgSO_4$, 1.25 mM $CaCl_2$, 136 mM NaCl, and 4.7 mM KCl). Glucose uptake was determined by incubation with KRPH buffer (pH 6.8) containing 50 μM $^3$H-labeled 2-deoxy-D-glucose (5–10 Ci/mmol; NET328A250UC, lot no. 1684189, PerkinElmer) for 30 min. Then, the cells were washed with KRPH buffer and solubilized in 1% Triton X-100. The radioactivity incorporated into the cells was measured with a liquid scintillation counter.

To evaluate SLC45A1 abundance after transfection, we performed western blots. Transfected COS-7 cells were lysed and separated for membrane fraction. Equal amounts of proteins from membrane fractions were resolved by SDS-PAGE, transferred to a nitrocellulose filter, and immunoblotted with an anti-SLC45A1 antibody (1:1,000; ab123314, lot no. GR86331-1, Abcam). We re-probed blots with an anti-β-actin antibody (1: 1,000; 4967L, Cell Signaling Technology) to monitor the quantity and integrity of cell-surface protein. SLC45A1 and β-actin were detected by chemiluminescence with an ECL system (GE Healthcare) and visualized with a Lumino Graph imaging analyzer (ATTO). These experiments showed that the levels of SLC45A1 variants were similar to wild-type (WT) levels, suggesting that none of the variants tested affect the stability of the protein (Figure 2A).

The accumulation of WT SLC45A1 in COS-7 cells led to 4.5-fold more glucose transport activity than in mock-transfected cells (Figure 2B). The glucose transport activity of the p.Arg176Trp and p.Ala210Val SLC45A1 variants was significantly decreased by approximately 50% ($p = 0.013$) and 33% ($p = 0.008$), respectively, in comparison with that of intact SLC45A1. These results indicate that Arg176 and Ala210 are critical for the glucose transport activity of SLC45A1. Anazi et al.[10] recently reported a homozygous c.167T>C (p.Ile56Thr) missense mutation in *SLC45A1* (or c.269T>C [p.Ile90Thr]; GenBank: NM_001080397.2)

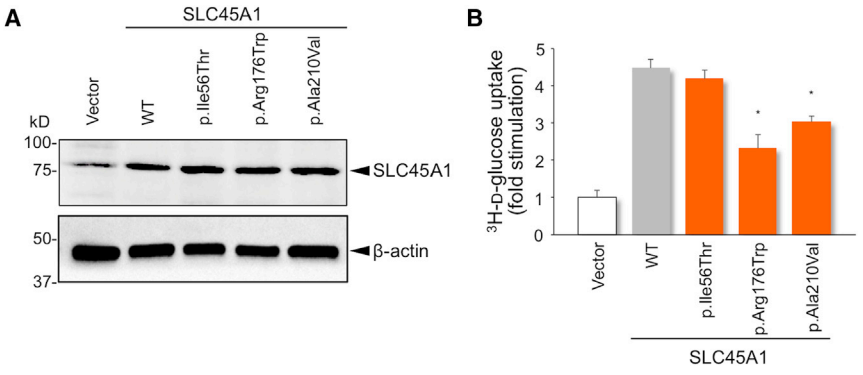


**Figure 2. Effect of SLC45A1 Variants on SLC45A1 Glucose Transport Activity**

(A) WT and variant SLC45A1 abundance. Lysates of membrane fraction (20 μg protein/lane) from COS-7 cells transfected with the indicated plasmid were separated by 7% SDS-PAGE and immunoblotted with antibodies against SLC45A1 (top) as described in Simpson et al.[9] Equal protein loading was confirmed by re-blotting with anti-β-actin antibodies (bottom). The positions of standard molecular masses (in kD) are indicated on the left.

(B) Inhibition of glucose uptake of COS-7 cells transfected with SLC45A1 variants. Data on glucose uptake are expressed as fold stimulation in relation to the extent of uptake observed with COS-7 cells transfected with a blank vector (white bar). Results are presented as mean ± SEM of three individual experiments (n = 6 per plasmid per each experiment). Statistical significance was calculated by ANOVA (*$p < 0.05$ in comparison with glucose uptake to WT SLC45A1). Post hoc comparison was performed with a Sidak test.

in a 5-year-old girl with developmental delay, ataxia, and Dandy-Walker malformation. Although this phenotype somewhat overlaps that of our affected individuals, none of our individuals had cerebellar abnormalities. Unlike the variants we reported herein, which lie in the boundaries of the transmembrane regions of SLC45A1, the p.Ile56Thr variant is located the N-terminal intracellular domain. Moreover, we found that this variant had no significant effect on the glucose transport activity of SLC45A1 (Figure 2). Thus, p.Ile56Thr either is not pathogenic or affects the function of the protein in a manner that is not measured by our assay.

Combined with functional evidence that these mutations affect protein function, the presence of rare homozygous variants segregating in affected members of two unrelated families with similar phenotypes strongly suggests that the variants identified in *SLC45A1* are pathogenic. The main clinical features of the four affected individuals are moderate to severe ID, epilepsy, and associated neuropsychiatric features (see Table 1 for a summary of clinical features). Indeed, three individuals have neuropsychiatric disorders, and the fourth individual is too young for symptoms to be apparent (11 months). Two individuals have autistic traits, and two have varying degrees of anxiety and obsessive-compulsive traits. Extra-neurologic features were observed: cardiac abnormalities were noted in two individuals (coarctation of the aorta and PDA), and renal abnormalities were noted in two siblings (renal agenesis and nephrocalcinosis). It is unclear at this time whether these features are related to *SLC45A1* mutations. Interestingly, bilateral nephrocalcinosis has been previously observed in one individual with epileptic encephalopathy associated with a de novo mutation in *SLC1A2*.[11] Of note, the individuals from family B, who display more severe developmental delay and ID, carry a missense mutation that results in a greater impairment of glucose transport, as measured in our in vitro assay (Figure 2B).

*SLC45A1* was initially identified in the context of a screen for genes upregulated by hypercapnia in the ventral medullary surface (VMS) of the medullary oblongata.[3] The VMS is the site of chemosensitive neurons that sense acidosis and hypercapnia and induce hyperventilation. In vitro, SLC45A1 possesses glucose transport activity that is enhanced by acidification. All together, these observations suggest that SLC45A1 might play a role in the regulation of respiration, possibly by controlling energy production in VMS neurons. Additional work would be needed to further confirm these findings. Although our affected individuals did not show any gross abnormalities of breathing, we cannot exclude the possibility that they display subtle aberrations of respiratory control. SLC45A1 also localizes to the cortex. Its function in this part of the brain remains unknown. However, it appears likely that SLC45A1 also functions as a glucose transporter in this area of the brain in view of its structure and activity in transfected cells.

The *SLC45A1* mutations identified in both families appear to be hypomorphic given that they resulted in a reduction but not abolition of intracellular glucose transport by SLC45A1 in our in vitro assay. It is tempting to speculate that recessive mutations causing a more severe dysfunction of the transporter would not be compatible with life or would result in a different and more dramatic phenotype. Heterozygous variants in the cerebral glucose transporter GLUT1 (or SLC2A1) also cause ID and epilepsy.[2,12,13] Variants in the GLUT1 transporter appear to be haploinsufficient or hypomorphic in that they decrease glucose uptake in erythrocytes to 37%–72% of that of controls.[14] There is a correlation between the decrease in glucose uptake and clinical severity.[14] Complete loss of GLUT1 function has not been reported in humans. As we postulated for SLC45A1, a more severe reduction of GLUT1-dependent glucose transport might likewise compromise postnatal survival.

The classic features of cerebral GLUT1 transporter deficiency include a combination of early-onset drug-resistant infantile seizures, neurodevelopmental delay, acquired microcephaly, complex movements disorders, spasticity, and ataxia[15] (also see GeneReviews in the Web Resources). The phenotypic spectrum of GLUT1 transporter deficiency

**Table 1. Clinical Features of Individuals with Mutations in *SLC45A1***

| | Family A | | Family B | |
|---|---|---|---|---|
| | **II-2** | **II-3** | **II-3** | **II-4** |
| *SLC45A1* mutation | c.629C>T (p.Ala210Val) | c.629C>T (p.Ala210Val) | c.526C>T (p. Arg176Trp) | c.526C>T (p. Arg176Trp) |
| Age | 22 years | 20 years | 8 years | 11 months |
| Gender | female | female | male | male |
| Microcephaly | no | no | no | no |
| GDD or ID | moderate ID, verbal | moderate ID, verbal | severe ID, nonverbal | GDD |
| Psychiatric features | OCD traits, anxiety | OCD, autistic traits, anxiety | autistic traits | NA |
| Dysmorphisms | downslanting eyes, smooth philtrum, thin upper lip | downslanting eyes, smooth philtrum, thin upper lip | downslanting eyes, triangular face, smooth philtrum, thin lips | flat, triangular face, smooth philtrum, thin lips, widely spaced eyes, depressed nasal bridge, facial hemangioma |
| Brain MRI | normal | normal | normal | not done |
| Epilepsy, type, onset | yes (temporal lobe seizures at 4.5 years) | yes (temporal lobe seizures at 11 years) | yes (febrile and secondarily generalized afebrile seizures at 7 months) | no |
| Other | patent ductus arteriosus | – | hypercalcemia, failure to thrive, agenesis of right kidney | failure to thrive, coarctation of the aorta, nephrocalcinosis |

Abbreviations are as follows: GDD, global developmental delay; ID, intellectual disability; MRI, magnetic resonance imaging; NA, not applicable; and OCD, obsessive-compulsive disorder.

has expanded with the recognition of variants causing, for example, predominant ataxia or dystonia without seizures, paroxysmal exercise-induced dyskinesia with or without epilepsy, and a spectrum of epilepsy syndromes such as early-onset absence epilepsy, myoclonic astatic epilepsy, focal seizures, and infantile spasms.[16] (also see GeneReviews in the Web Resources). Our affected individuals share clinical features associated with the GLUT1-deficiency phenotype, such as focal epilepsy and developmental delay or ID; however, they lack many features associated with the classic GLUT1 deficiency (such as a dystonia, ataxia, and microcephaly) or non-classic GLUT1-deficiency phenotype (such as paroxysmal movement disorder, early-onset absence epilepsy, or myoclonic astatic epilepsy). Furthermore, the hypoglycorrhacchia observed in over 90% of individuals with GLUT1 deficiency, characterized by low CSF glucose (<60 mg/dL) and a low ratio of CSF to serum glucose (<0.4),[4] was not documented in the one individual who was tested from our cohort. This individual had normal CSF glucose and a normal ratio of CSF to serum glucose (0.7).

The ketogenic diet is a specific and effective treatment for *GLUT1*-related epilepsy, given that ketone bodies use a different transporter to cross the BBB and thus provide the brain with the only known fuel alternative to glucose for metabolism. The administration of a modified Atkins diet for 6 months at the age of 16 years did not improve the control of seizures in individual A.II-3 or her behavior. Nevertheless, the effectiveness of the ketogenic and/or modified Atkins diet in individuals with *SLC45A1* mutations warrants further investigation because it could represent a potential specific treatment.

In summary, we have identified homozygous missense mutations in the cerebral glucose transporter *SLC45A1* in four individuals with moderate to severe ID, epilepsy, and neuropsychiatric features from two families. We provide functional evidence that these missense mutations result in decreased intracellular glucose transport by SLC45A1. Together, our data suggest that autosomal-recessive mutations in *SLC45A1* result in ID and epilepsy. SLC45A1 is thus the second cerebral glucose transporter, in addition to GLUT1, to be involved in human disease and implicated in neurodevelopmental disability. Identification of additional individuals with mutations in *SLC45A1* will allow better definition of the associated phenotypic spectrum and exploration of potential targeted treatment options.

## Conflicts of Interest

The Department of Molecular and Human Genetics at Baylor College of Medicine receives revenue from clinical testing, including exome sequencing, performed at Baylor Genetics Laboratories.

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## Web Resources

1000 Genomes Project, http://browser.1000genomes.org/index.html
ExAC Browser, http://exac.broadinstitute.org/
GATK Best Practices, http://www.broadinstitute.org/gatk/guide/topic?name=best-practices
GenBank, https://www.ncbi.nlm.nih.gov/genbank/
GeneReviews, Wang, D., Pascual, J.M., and De Vivo, D. (1993). Glucose Transporter Type 1 Deficiency Syndrome, https://www.ncbi.nlm.nih.gov/books/NBK1430/
NCBI HomoloGene, http://www.ncbi.nlm.nih.gov/homologene
NHLBI Exome Sequencing Project (ESP) Exome Variant Server, http://evs.gs.washington.edu/EVS/
OMIM, http://www.omim.org
PolyPhen-2, http://genetics.bwh.harvard.edu/pph2/
SIFT, http://sift.jcvi.org/
The Human Protein Atlas, http://www.proteinatlas.org/
UniProt (SLC45A1), http://www.uniprot.org/uniprot/Q9Y2W3

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