

Supplemental Data

Mutations in the Na⁺/Citrate Cotransporter NaCT (SLC13A5) in Pediatric Patients with Epilepsy and Developmental Delay

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Supplementary Table S1. Demographic information and birth history for subjects in current study.

Family	Subject	Preterm	Country of origin	Ethnicity	Maternal education	Paternal education	Consanguinity
A	1	No	USA	Caucasian	Bachelor's	Doctorate	No
	2	No					
B	3	No	Netherlands	Caucasian	Trade/Technical school	Trade/Technical school	No
C	4	No	USA	Caucasian	Bachelor's	Master's	No
	5	No					
D	6	Yes	USA	South East Asian	Bachelor's	Master's	No
E	7	Yes	Brazil	Hispanic/Latin American	Bachelor's	Bachelor's	No
	8	No					
F	9	No	Brazil	Caucasian	Master's	High school	No

Supplementary Table S2. Additional information on seizure history.

Subject	Status epilepticus (duration of longest episode if reported)	Seizure triggers	EEG findings	Medication trials, ineffective	Supplements/other treatments, ineffective (<u>underlined</u> if worsened seizures)
1	Y	Early morning Fasting	Focal sharps Generalized spike-wave Normal background	Clobazam	<u>Atkins diet</u> Carnitine Cornstarch Coenzyme Q10 Folic acid P5P <u>Triheptanoïn</u>
				Clonazepam	
				Ethosuximide	
				IVIG	
				Lacosamide	
				Lamotrigine	
				Levetiracetam	
				Levodopa-carbidopa	
				Lorazepam	
				Oxcarbazepine	
				Primidone	
				Steroids	
				Topiramate	
				Valproic acid	
Zonisamide					
2	Y	None known	Focal spikes Excess beta	Levetiracetam	Carnitine Folic acid P5P Triheptanoïn
				Lorazepam	

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Supplementary Table S2. *Continued.*

3	Y (1 wk)	Fever Fasting Nighttime Sleep transitions	NR	Clobazam Clonazepam Levetiracetam Valproic acid Vigabatrin	P5P Pyridoxine
4	Y (1.5 h)	Fever Fasting	Right frontal spikes	Carbamazepine Diazepam Lacosamide Lamotrigine Levetiracetam Oxcarbazepine Phenobarbital Phenytoin Vigabatrin Zonisamide	Epilepsy surgery Folic acid Pyridoxine
5	Y (40 min)	Fever Fear	Focal spikes associated with sleep	Carbamazepine Diazepam Levetiracetam Lorazepam Oxcarbazepine Phenobarbital Phenytoin Topiramate Valproic acid	Folic acid P5P Pyridoxine
6	Y (40 min)	Excitement Fatigue Nighttime	Most recent EEG normal	Diazepam Levetiracetam Levodopa-carbidopa Oxcarbazepine Phenobarbital Topiramate	Biotin Carnitine Folic acid Pyridoxine
7	Y	None known	Interictal epileptiform activity higher in centrotemporal regions, left > right, especially during sleep	Carbamazepine Ethosuximide Gabapentin Levetiracetam Oxcarbazepine Phenobarbital Vigabatrin Zonisamide	Carnitine Folic acid Vitamin C
8	Y (15 min)	None known	Irritability in right centrotemporal and left temporal areas	Carbamazepine Levetiracetam Oxcarbazepine Vigabatrin Zonisamide	Folic acid Pyridoxine Vitamin C Vitamin E
9	Y (30 min)	Fever Loud noises Agitation	Focal seizures	Carbamazepine Diazepam Felbamate Lacosamide Levetiracetam Phenobarbital Valproic acid	Arginine Cannabidiol Carnitine Folic acid Pyridoxine Vitamin B1 Vitamin B2 Vitamin B5 Vitamin C Vitamin D

Abbreviations are as follows: NR, not reported; Y, yes; P5P, pyridoxal-5-phosphate.

Supplementary Table S3. Additional information on developmental history.

Subject	Age (years)	Motor development	Language development	Social development	Developmental regression
1	11.8	Sat at 8 months Walked at 4 years Uses adaptive bicycle	First words at 10 months Follows complex commands	Happy disposition Good social skills	Language loss between 2-4 years, regained some language after improved seizure control
2	2	Sat at 10 months Able to crawl and cruise Uses a walker	First words at 20 months Good receptive language Uses sign language, gestures and communication device	Happy disposition Good social skills	None
3	2.9	Unable to sit independently Non-ambulatory	Does not speak Good receptive language Uses eyes to communicate Arms/Hands too weak to use communication device	Good sense of humor Good social skills	Lost ability to coo and roll at 2 years 10 months
4	5.7	Unable to sit independently Non-ambulatory Uses wheelchair	Does not speak Understands some words and commands Does not use communication device	Good social skills	None
5	8.7	Sat at 1 year Able to stand independently Uses wheelchair, walker and stander	Does not speak Understands some words Uses eyes to communicate	Throws tantrums when upset	None
6	9.6	NR	NR	NR	NR
7	18.7	Sat at 2 years Takes steps in therapy with orthotics and assistance, can not walk independently Uses wheelchair	First words at 4 years Good receptive language Uses communication device	Good social skills Good listening and emotional skills	None
8	15.5	Sat at 1 year Walked at 7 years Does not require assistive devices	Does not speak Good receptive language Uses gestures and communication device	NR	None
9	4	Unable to sit independently Non-ambulatory	Does not speak Good receptive language Does not use communication device	Happy disposition	Previously able to sit independently

Abbreviation: NR, not reported.

Supplementary Table S4. Effect of culture treatments or additives on citrate transport activity in wild type and mutant NaCT.

Treatment	Concentration (mM) or time	Effect on WT	Effect on Mutants	Reference for dosage or approach
Culture treatments:				
Cold (28°C)	42 h	Decrease 23%	No G219R activity	(1)
Acetazolamide (AZ)	0.50	None	None	(2)
4-Aminopyridine (4AP)	0.25	Decrease	None	(3)
Glycerol	0.50	Decrease	None	(4)
Heptanoic Acid (HA)	5.0	None	Not tested	(5)
4-Phenylbutyric acid (4PBA)	1.0	None	None	(6)
Sebacic acid (C10)	0.5	Decrease 50%	None	(7)
Assay treatments:				
Heat shock (45°C)	15 min	Decrease	None	(8)
Methotrexate	5 mM	Increase 28%	None	(9)

COS-7 cells cultured in 24 well plates were transfected as described in Materials and Methods. The medium additives or change in culture temperature to 28°C were started 6 h after transfection. Transport of 100 μ M 14 C-citrate was measured 42 h later, as described for Figure 1. Heat shock 45°C was applied for 15 min followed by a 1 h rest at 37°C. Methotrexate was added to the transport medium.

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