

Prevalence of Genetic Disorders and GLUT1 Deficiency in a Ketogenic Diet Clinic

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ABSTRACT: Between July of 2012 and December of 2014, 39 patients were enrolled prospectively to investigate the prevalence of glucose transporter 1 (GLUT1) deficiency in a ketogenic diet clinic. None of them had GLUT1 deficiency. All patients seen in the same clinic within the same period were reviewed retrospectively. A total of 18 of these 85 patients had a genetic diagnosis, including GLUT1 deficiency, pathogenic copy number variants, congenital disorder of glycosylation, neuronal ceroid lipofuscinosis type II, mitochondrial disorders, tuberous sclerosis, lissencephaly, and *SCN1A*-, *SCN8A*-, and *STXBP1*-associated epileptic encephalopathies. The prevalence of genetic diagnoses was 21% and prevalence of GLUT1 deficiency was 2.4% in our retrospective cohort study.

RÉSUMÉ: **Prévalence de maladies génétiques et du syndrome du déficit en transporteur de glucose de type 1 au sein d'une clinique utilisant le régime céto-gène.** De juillet 2012 à décembre 2014, 39 patients fréquentant une clinique utilisant le régime céto-gène ont été inclus de manière prospective dans cette étude afin d'analyser la prévalence du syndrome du déficit en transporteur de glucose de type 1 (GLUT1). Aucun d'entre eux ne souffrait d'un tel déficit au moment de son inclusion. De plus, tous les patients soignés au cours de la même période dans la même clinique ont ensuite fait l'objet d'une analyse rétrospective. Sur ces 85 patients, 18 ont reçu un diagnostic de maladie génétique, ce qui incluait le syndrome du déficit en GLUT1, une variabilité dans le nombre de copies de nature pathogène, des anomalies congénitales de la glycosylation, des céroïdes-lipofuscinoses neuronales de type II, des troubles mitochondriaux, la sclérose tubéreuse, la lissencéphalie et des encéphalopathies épileptiques causées par des mutations sur les gènes *SCN1A*, *SCN8A* et *STXBP1*. La prévalence de diagnostics génétiques a donc été de 21 % alors que la prévalence du syndrome du déficit en GLUT1 a été de 2,4 % dans le cadre de notre étude rétrospective de cohorte.

Keywords: Epilepsy genetics, Glucose transporter 1 deficiency, GLUT1, SLC2A1, Ketogenic diet

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Drug-resistant epilepsy is defined as the continuation of debilitating seizures, despite adequate therapy with two or more antiepileptic medications alone or in combination.¹ The most severe forms of drug-resistant epilepsy usually begin in infancy and are associated with global developmental delay, cognitive dysfunction, and ongoing epileptiform activity, which cause further cognitive slowing and decline, defined as epileptic encephalopathy.¹ Half of these children are candidates for ketogenic diet therapy for seizure management.

Ketogenic diets have been used since the 1920s for the treatment of intractable epilepsy.² There are only two inherited metabolic disorders for which the effect of the ketogenic diet on seizure control is well known: (1) glucose transporter 1 (GLUT1) deficiency, which is caused by likely pathogenic variants in *SLC2A1*,³ and (2) pyruvate dehydrogenase complex deficiency, which results in decreased acetyl-CoA and ATP production.⁴ In both disorders, a ketogenic diet provides an alternative fuel source to the brain and bypasses glucose metabolism.

Discontinuation of the ketogenic diet will certainly result in seizure reoccurrence and deterioration of cognitive functions in patients with GLUT1 deficiency. Confirmed diagnosis of GLUT1 deficiency will likely prevent the negative effects of early

discontinuation of the ketogenic diet. To identify patients with GLUT1 deficiency and be able to decide on the duration of the ketogenic diet therapy, we performed a prospective cohort study to investigate GLUT1 deficiency by targeted *SLC2A1* genetic testing using direct sequencing and multiplex ligation probe amplification (MLPA) of *SLC2A1* in patients with drug-resistant epilepsy treated with the ketogenic diet. To investigate the prevalence of GLUT1 deficiency and other genetic diagnoses in epilepsy patients on the ketogenic diet, we also retrospectively

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reviewed patients for their diagnosis in the same clinic within the same period as our prospective cohort study.

The prevalence of GLUT1 deficiency prospective cohort study was approved by the institutional research ethics board (no. REB#1000033443). All patients with drug-resistant epilepsy on the ketogenic diet with no genetic diagnosis were approached for this prospective cohort study between July of 2012 and December of 2014 at a single ketogenic diet clinic. DNA samples of enrolled patients with signed consents underwent direct sequencing (all exons and intron boundaries), and MLPA of the *SLC2A1* for deletions and duplications at the Metabolic Laboratory in Amsterdam (the Netherlands) according to their standard diagnostic laboratory methods.

The prevalence of genetic diagnoses in patients with drug-resistant epilepsy on the ketogenic diet as a retrospective cohort study for the patients seen between July of 2012 and December of 2014 at the same ketogenic diet clinic was also approved by the institutional research ethics board (no. REB#1000049758). We reviewed electronic patient charts for clinical features, neuroimaging studies, biochemical investigations, and molecular genetic investigations, including targeted next-generation sequencing panels of epileptic encephalopathy. All information was entered into an Excel database.

Thirty-nine patients underwent prospective targeted direct sequencing and MLPA of *SLC2A1*. None of these patients had a pathogenic variant in *SLC2A1*.

There were 99 patients in the retrospective cohort study, including the 39 patients from the prevalence of GLUT1 deficiency prospective cohort study. There were 37 females and 62 males. The current average age was 8 years and 10 month \pm 5 years and 5 month standard deviation (range 1 year and 6 months-18 years). The average age of seizure onset was 20.14 \pm 30.83 months (range = 1-168 months).

The clinical features, seizure types, number of antiepileptic medications, and neuroimaging features are summarized in Table 1. The most common seizure type (74%) was generalized tonic-clonic (Table 1). Fifty percent of patients had more than one type of seizure, 7% had a movement disorder, and 13% had a behavioral disorder.

The majority of patients (89%) had generalized spike or spike-and-wave electrical activity in their electroencephalography (EEG). Eighty-nine percent of patients underwent brain magnetic resonance imaging (MRI). Brain MRI was abnormal in 66% of these patients (Table 1).

Eighty-nine percent of patients were tried on three or more antiepileptic medications prior to the ketogenic diet therapy. A small number of patients received more than 10 antiepileptic medications.

A total of 14 of the 99 patients had no genetic testing and also declined to participate in the prospective GLUT1 study. We excluded these patients from the prevalence calculations. Eleven patients were diagnosed by targeted genetic testing or microarray including GLUT1 deficiency ($n=2$, published previously^{5,6}), pathogenic copy number variants ($n=3$), congenital disorders of glycosylation ($n=1$), neuronal ceroid lipofuscinosis type II ($n=1$), mitochondrial disorder by mutations in the *NDUFS7* ($n=1$), tuberous sclerosis ($n=2$), and lissencephaly by mutation in the *LISI* ($n=1$) (Table 2). Seven patients were diagnosed by targeted next-generation sequencing panels of epileptic encephalopathy genes, including *SCN1A*- ($n=4$), *SCN8A*- ($n=1$), and *STXBPI*- ($n=2$) associated epileptic encephalopathies (Table 2).

Table 1: Clinical features and neuroimaging results of 99 patients on the ketogenic diet

	Distribution of clinical and neuroimaging features	Number of patients
Clinical features	Neurodevelopment:	
	GDD (from mild to profound) (88%)	87
	Learning disability (6%)	6
	Normal (6%)	6
	Seizure types:	
	GTC	73
	Absence	52
	Myoclonic	47
	Atonic	22
	Infantile spasms	12
	Partial	4
	Movement disorders (7%):	
	Dystonia	2
	Chorea	1
	Ataxia	3
	Dyskinetic movement disorder	1
Behavioral problems (13%):		
ASD	10	
ADHD	2	
Hyperactivity	1	
Brain MRI findings	Normal	34
	Cerebral atrophy	19
	Cerebellar atrophy	2
	Corpus callosum abnormalities (dysgenesis/agenesis/thin/thick)	8
	Hypoxic changes/gliosis	9
	Tubers	2
	Hypomyelination	2
	Low-grade tumor	1
	Focal or diffuse cortical dysplasia/heterotopia	5
	Diffuse brainstem and basal ganglia changes	6
	Not available	11
Antiepileptic medications	2	11
	≥ 3	88

ADHD = attention deficit hyperactivity disorder; ASD = autism spectrum disorder; GDD = global developmental delay; GTC = generalized tonic-clonic; MRI = magnetic resonance imaging.

The prevalence of a genetic diagnosis was 21% (18/85) in our retrospective cohort study.

In patients with drug-resistant epilepsy on the ketogenic diet, the prevalence of GLUT1 deficiency was 2.4%, and the prevalence of a genetic diagnosis was 21% in the single ketogenic diet clinic. The *SCN1A*-associated epileptic encephalopathy was the most common genetic diagnosis in our study. We did not identify any new patients

Table 2: All patients with a genetic diagnosis

Patient number/ sex/current age (y)/ diagnosis	Clinical features/age of onset/age of diagnosis	Genetic testing
1/F/19.5/GLUT1 deficiency ⁶	Cognitive dysfunction, ataxia, AbS, GTS/ 6 mo/ 11 y	Heterozygous de novo, known p.Arg330X in <i>SLC2A1</i>
2/M/12.5/GLUT1 deficiency ⁵	Cognitive dysfunction, Abs/ 2 y/ 11.5 y	Heterozygous de novo, known p.Ala275Thr in <i>SLC2A1</i> gene
3/F/3.5/ <i>ALG11</i> -CDG	GDD, hypotonia, CP, IS, GTCS, GTS/ 3 mo/ 4 y	Homozygous, novel p.I414N in <i>ALG11</i>
4/M/11/NCL type 2	GDD, ataxia, MS, AbS, GTS/ 48 mo/ 5 y	Compound heterozygous novel c.509-1G > C/ known p.Arg208X in <i>CLN2</i> *
5/M/17 y, 10 mo/Leigh's disease	GDD, hypotonia, GTCS, MS, scoliosis/ 2.5 y	Compound heterozygous novel p.Met1/ known p.Val122Met in <i>NDUFS7</i> **
6/M/12/pathogenic CNV	GDD, ASD, AS/ 120 mo/ 4 y	15q11.1-q13.2 (20,063,340-30,762,747)x3, 10.7 Mb triplication
7/F/15/pathogenic CNV	GDD, GTCS, AbS, / 4 mo/ 1 y	18q deletion syndrome (47, XX, del(18)(q11.1), + r(18)(p.11.1q23)
8/F/3/Aicardi syndrome and pathogenic CNV	GDD, hypotonia, IS/ 3 w 3.5 mo	Aicardi syndrome (clinical diagnosis) 16p13.11 (15,507,164-16,400,833) x1 (0.894 MB deletion)
9/F/4.5/TS	GDD, renal angioliopoma, AS, PS/ 7 mo/ 7 mo	TS clinical diagnosis <i>TSC1</i> and <i>TSC2</i> genetic tests negative
10/M/14/TS	GDD, ASD, IS, GTCS/ 2 mo/ 10 y	Heterozygous, de novo, novel c.1149_1155delCGTCCAT in <i>TSC2</i>
11/F/2.5/lissencephaly	GDD, IS, GTCS/ 3 mo/ 8 mo	Heterozygous de novo, novel c.1100delA in <i>LIS1</i>
12/F/11/ <i>SCN1A</i> -associated EE	GDD, MS, GTCS, AbS/ 4 mo/ 3 y	Heterozygous de novo, novel, p.Gln1036X in <i>SCN1A</i>
13/M/13/ <i>SCN1A</i> -associated EE	GDD, GTCS, MS, AbS, PS, AS/ 4 mo/ 9 y	Heterozygous de novo, novel p.T1458X in <i>SCN1A</i>
14/M/4/ <i>SCN1A</i> -associated EE	GDD, GTCS/ 5 mo/ 5 mo	Heterozygous, de novo known p.Arg1596Cys in <i>SCN1A</i> ***
15/M/4 y/ <i>SCN1A</i> -associated EE ⁵	GDD, AS, GTCS, MS/ 8 mo/ 2 y	Heterozygous de novo, known (p.Gln450X) in <i>SCN1A</i>
16/M/5/ <i>SCN8A</i> -associated EE	GDD, GTCS, MS/ 2 mo/ 4 y	Heterozygous de novo, novel p.Ile763Val in <i>SCN8A</i>
17/F/4/ <i>STXBPI</i> -associated EE ⁵	GDD, hypotonia, GTCS/ 2 w/ 3.5 y	Heterozygous de novo, known p.Ile539del in <i>STXBPI</i>
18/M/5/ <i>STXBPI</i> -associated EE	GTCS, GDD, ASD/ 1.5 y / 4 y	Heterozygous de novo, novel, p.Met252Thr in <i>STXBPI</i>

*Sleat DE, Donnelly RJ, Lackland H, et al. Association of mutations in a lysosomal protein with classical late-infantile neuronal ceroid lipofuscinosis. *Science*. 1997;277(5333):1802-5.

**Triepeles RH, Van den Heuvel LP, Loeffen JL, et al. Leigh syndrome associated with a mutation in the *NDUFS7* (PSST) nuclear encoded subunit of complex I. *Ann Neurol*. 1999;45(6):787-90.

***Harkin LA, McMahon JM, Iona X, et al. The spectrum of *SCN1A*-related infantile epileptic encephalopathies. *Brain*. 2007;130(Pt 3):843-52.

AbS = absence seizures; ADHD = attention deficit hyperactivity disorder; AS = atonic seizures; ASD = autism spectrum disorder; CDG = congenital disorders of glycosylation; CNV = copy number variants; CP = cerebral palsy; EE = epileptic encephalopathy; F = female; GCS = generalized clonic seizures; GDD = global developmental delay; GTCS = generalized tonic-clonic seizures; GTS = generalized tonic seizures; GLUT1 = glucose transporter 1; IS = infantile spasms; M = male; mo = month(s); MS = myoclonic seizures; PS = partial seizures; TS = tuberous sclerosis; w = week(s); y = year(s).

with GLUT1 deficiency, which could be due to the small number of patients enrolled in the prospective cohort study.

The prevalence of GLUT1 deficiency in epilepsy patients on the ketogenic diet was between 0 and 8.5% in three studies.⁷⁻⁹ Those studies included a total of 307 patients (274 using direct sequencing only and 33 direct sequencing and MLPA of *SLC2A1*).⁷⁻⁹ Only one patient was diagnosed with GLUT1 deficiency.⁹ Including our prospective study results, so far 346 patients have undergone targeted *SLC2A1* genetic testing, and the prevalence of GLUT1 deficiency was 0.29% in patients with drug-resistant epilepsy on the ketogenic diet. There is only one retrospective study that looked at the frequency of GLUT1 deficiency in patients on the ketogenic diet.⁸ That study reported occurrence of GLUT1 deficiency in 11 of 130 patients (8.5%). Including our retrospective study results, so far 13 of 215 patients with drug-resistant epilepsy on the ketogenic diet (6%) were diagnosed with GLUT1 deficiency.⁸ Comparison of the prospective (0.29%) and retrospective (6%) prevalence data of GLUT1 deficiency raises the question of whether GLUT1

deficiency is a rare disease. To investigate the prevalence of GLUT1 deficiency in patients with drug-resistant epilepsy on the ketogenic diet, we should be enrolling larger numbers of patients. However, we think that the small number of studies (including our current study) gives valuable and sufficient information for the prevalence of GLUT1 deficiency in patients with drug-resistant epilepsy on the ketogenic diet.

There are only two studies reporting a 14% prevalence of genetic diagnoses in patients on the ketogenic diet.^{7,9} In one of those studies, *SCN1A*-associated epileptic encephalopathy was the most common genetic diagnosis in 5.6% of patients.⁹ Our study reports a 21% prevalence of a genetic diagnosis in the ketogenic diet clinic. *SCN1A*-associated epileptic encephalopathy was the most common genetic diagnosis (22%) in our retrospective cohort study. Targeted next-generation sequencing panels of epileptic encephalopathy genes confirmed a genetic diagnosis in 39% of the patients in our study, which is the likely cause of the slightly higher prevalence of genetic diagnoses in our study. Despite the fact that *SLC2A1* is included in the targeted

next-generation sequencing panels of epileptic encephalopathy genes, we did not identify any patients with GLUT1 deficiency in 93 patients who underwent this genetic testing in our recent study.⁵ We think that GLUT1 deficiency might likely be a rare genetic disease.

Good responses to the ketogenic diet raises a suspicion of GLUT1 deficiency.^{3,10} Seizure freedom was reported in 67% of the patients (41/61) with GLUT1 deficiency in one study.¹⁰ In another study, seizure freedom was found in 90% of patients (10/11) with GLUT1 deficiency, whereas only 9% of the responders with no diagnosis were seizure-free.⁷ The authors concluded that seizure freedom is an important clue to the diagnosis of GLUT1 deficiency. However, another study reported <8% seizure freedom in patients on the ketogenic diet.⁹ The authors did not think that seizure freedom was solely related to the GLUT1 deficiency.⁹ In our study, both patients with GLUT1 deficiency, treated after 10 years of age, did not become seizure-free. One patient with no response had compliance problems,⁶ and another patient with good compliance had occasional seizures. It is not clear if a response to the ketogenic diet is solely dependent on compliance or that there are other factors that influence response to the ketogenic diet in patients with GLUT1 deficiency.

In summary, we report a wide spectrum of genetic disorders in patients with drug-resistant epilepsy on the ketogenic diet. The prevalence of genetic diagnoses was 21%, and the prevalence of GLUT1 deficiency was 2.4% in patients with drug-resistant epilepsy on the ketogenic diet. We believe that targeted next-generation sequencing panels of epileptic encephalopathy genes and clinical whole exome sequencing will likely increase the number of patients with GLUT1 deficiency to be treated with the ketogenic diet.

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DISCLOSURES

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Kobayashi, Gajja S. Salomons, and Saadet Mercimek-Andrews hereby declare that they have no conflicts of interest to disclose.

STATEMENT OF AUTHORSHIP

SH was responsible for patient enrollment, data analysis, arrangement of research samples, and drafting and finalizing of the manuscript. LB, JP, and EI were responsible for data entry and data analysis. MFO was responsible for direct sequencing and MLPA of *SLC2A1* for the research study. MZ, EJD, and JK were responsible for application of the ketogenic diet, patient enrollment, and revision and final approval of the manuscript. GSS was responsible for analysis of the results of direct sequencing and MLPA of *SLC2A1* for the research study, and revision and final approval of the manuscript. SMA was responsible for design and conceptualization of the study, analysis and interpretation of the data, and drafting and finalizing the manuscript for intellectual content.

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