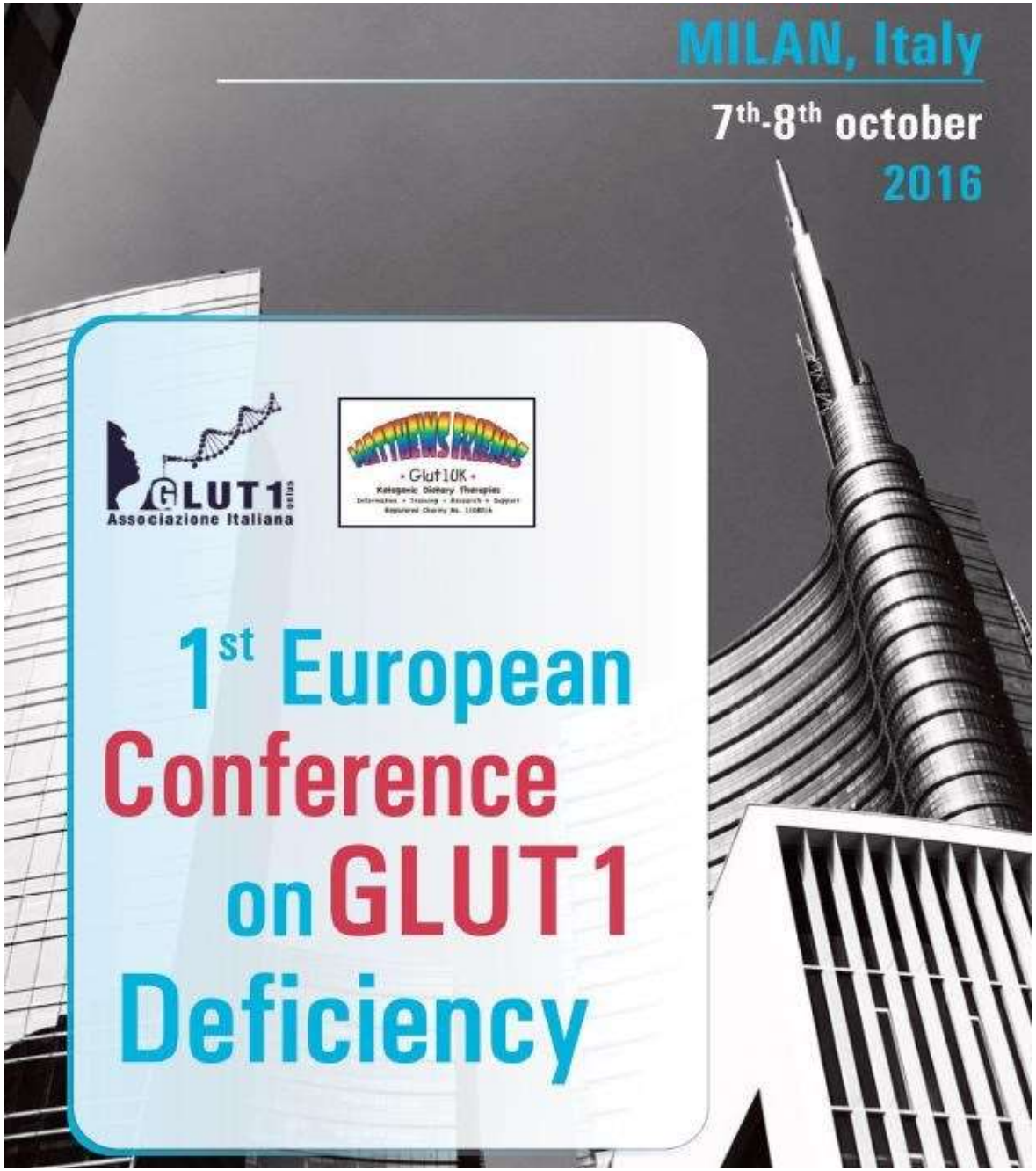


GLUT1 DS:
The Genetic Approach

Carla Marini, MD, PhD

Child Neurology Unit – AUO

Meyer Children's Hospital
Firenze – Italy

Outline

- Treatable, highly ***epileptogenic***, encephalopathy
- SLC2A1 or GLUT1 gene
- Inheritance
- Genotype
- Phenotype
- Genotype-phenotype correlations

SLC2A1 gene

SOLUTE CARRIER FAMILY 2 (FACILITATED GLUCOSE TRANSPORTER), MEMBER 1; SLC2A1

Alternative titles; symbols
GLUCOSE TRANSPORTER 1; GLUT; GLUT1
ERYTHROCYTE/HEPATOMA GLUCOSE TRANSPORTER

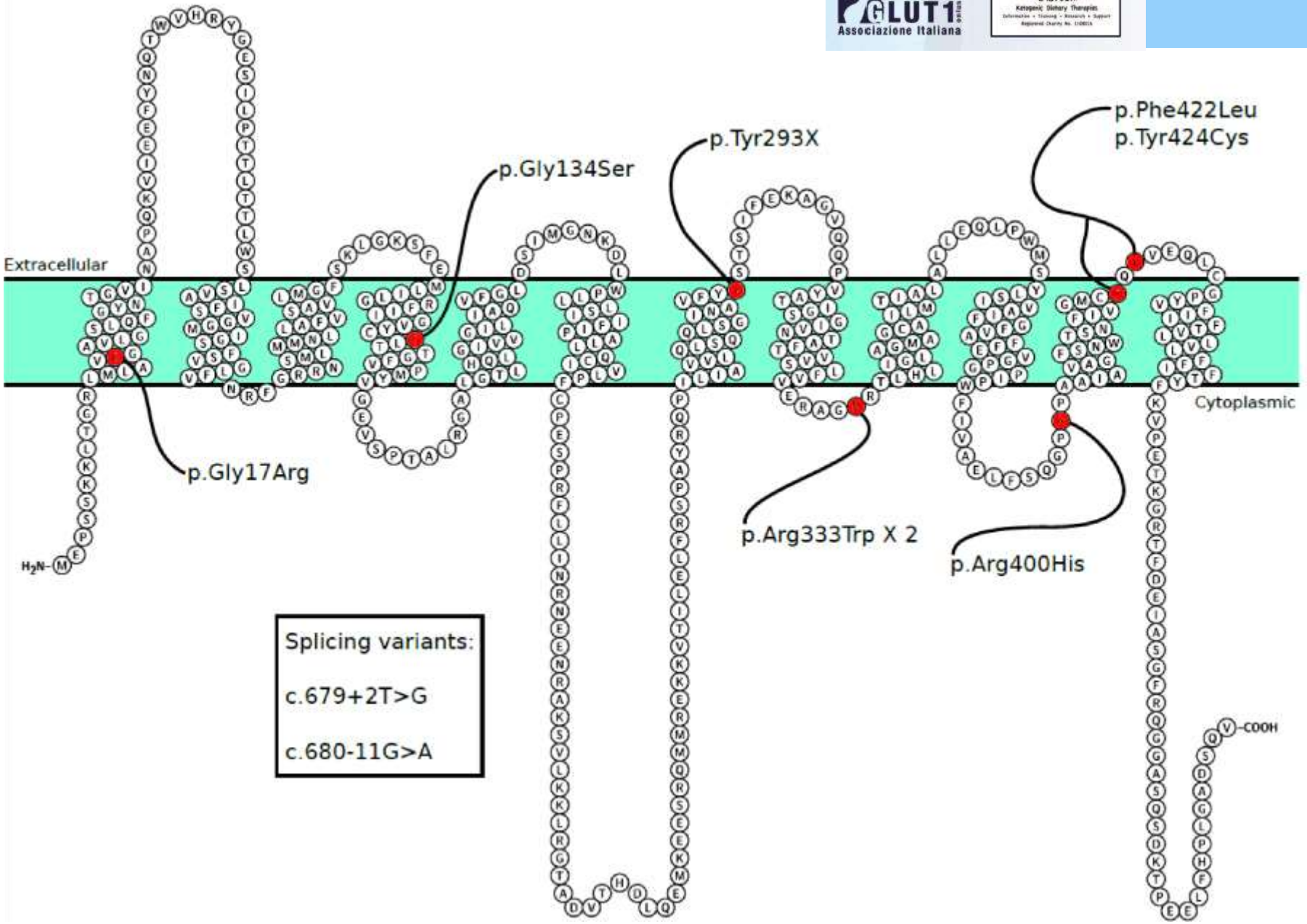
HGNC Approved Gene Symbol: SLC2A1

Cytogenetic location: 1p34.2 ***Genomic coordinates (GRCh38): 1:42,925,374-42,959,175*** (from NCBI)

Gene-Phenotype Relationships

Location	Phenotype	Phenotype MIM number	Inheritance (in progress)	Phenotype mapping key
1p34.2	Dystonia 9	601042	AD	3
	GLUT1 deficiency syndrome 1, infantile onset, severe	606777	AD, AR	3
	GLUT1 deficiency syndrome 2, childhood onset	612126	AD	3
	Stomatin-deficient cryohydrocytosis with neurologic defects	608885	AD	3
	{Epilepsy, idiopathic generalized, susceptibility to, 12}	614847	AD	3

Humans have 2 copies of the gene
Maternal copy + paternal copy



Mutations might be:

- Missense
- Frameshift
- Non sense
- Truncating
- deletions

How should the gene be analyzed?

- Sanger-based automated sequencing ⟶ to uncover missense or truncating mutations
- Sequencing must include regulatory sequences of the SLC2A1 gene, e.g. promoter sequences and/or sequences deep in introns
- About 10% of GLUT1-DS might also be caused by (partial) deletion of the SLC2A1 gene.
- Multiplex Ligation-dependent Probe Amplification (MPLA) must be performed in patients with suspected GLUT1 deficit

Inheritance

1st European Conference on Glut1 Deficiency

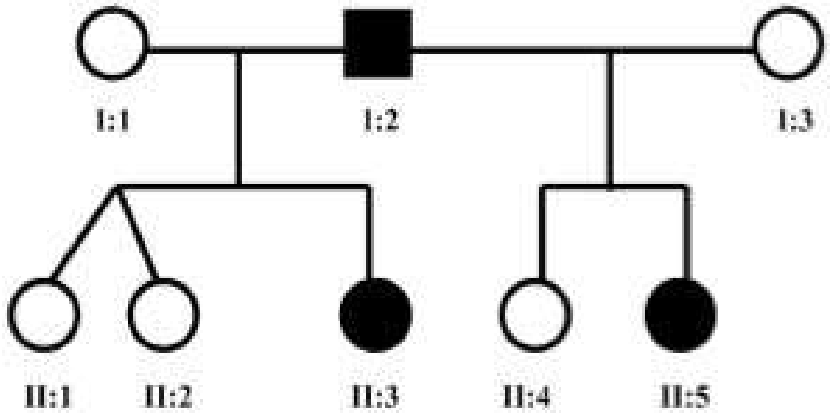
Milan, Italy
7th-8th October 2016

Human Molecular Genetics, 2001, Vol. 10, No. 1 63–68

Autosomal dominant transmission of GLUT1 deficiency

Jörg Klepper[1,+,§], Michèl Willemsen[2,+], Aad Verrips[2], Elena Guertsen[1], Ralf Herrmann[1], Christian Kutzick[1], Anne Flörcken[1] and Thomas Voit[1]

- Several affected family members over different generations
- Mutations are transmitted by mildly affected paren
- Mosaic mutations in the transmitting parent
- Non penetrant transmitting parent



Autosomal dominant Glut-1 deficiency syndrome and familial epilepsy

Brockmann K, Wang D, Korenke CG, von Moers A, Ho YY, Pascual JM, Kuang K, Yang H, Ma L, Kranz-Eble P, Fischbarg J, Hanefeld F, De Vivo DC.

Ann Neurol 2001;50(4):476-85.

Inheritance

Neuropediatrics. 2009 Oct;40(5):207-10. doi: 10.1055/s-0030-1248264. Epub 2010 Mar 10.

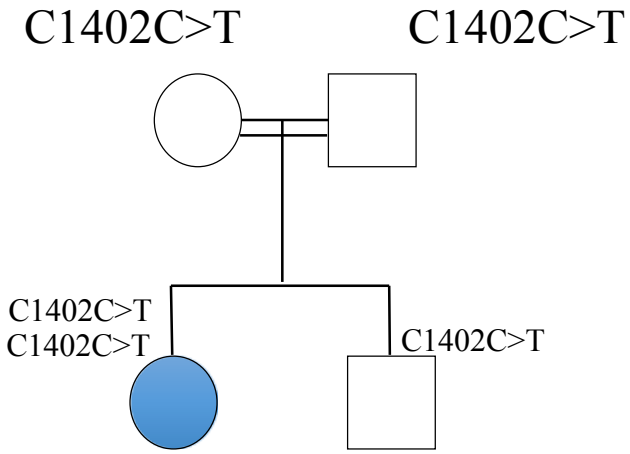
Autosomal recessive inheritance of GLUT1 deficiency syndrome.

Klepper J[1], Scheffer H, Elsaid MF, Kamsteeg EJ, Leferink M, Ben-Omran T.

Author information

Abstract

GLUT1 deficiency syndrome (GLUT1DS) is understood as a monogenetic disease caused by heterozygous SLC2A1 gene mutations with autosomaldominant and sporadic transmission. We report on a six-year-old girl from an inbred Arab family with moderate global developmental delay, epilepsy, ataxia, hypotonia, and hypoglycorrhachia (CSF glucose 36 mg/dL; CSF lactate 1.09 mmol/L; CSF/blood glucose ratio 0.44). Molecular analysis of the SLC2A1 gene identified a novel homozygous c1402C>T (p. Arg468Trp) mutation in exon 10 in the index patient and her asymptomatic younger sister. The mutation was absent in 120 control alleles of healthy individuals as well as in 400 alleles of other GLUT1DS patients. Arg468 represents a highly conserved, functionally important amino acid residue in the GLUT1 carboxy-terminus essential for substrate recognition and transport. Both unaffected parents were heterozygous for the mutation. A younger brother and two family members were healthy and carried the GLUT1 wild type. A ketogenic diet effectively controlled seizures in the index patient. We conclude that GLUT1DS can be transmitted as an autosomal recessive disease and provide new insights into genetic counselling for this treatable disorder.



Inheritance

Glut1 Deficiency: Inheritance Pattern Determined by Haploinsufficiency

Michael Rotstein, MD[1], Kristin Engelstad, BS[1], Hong Yang, MD[1], Dong Wang, MD[1], Brynn Levy, M.Sc.(Med)., Ph.D[2], Wendy K. Chung, MD[3], and Darryl C. De Vivo, MD.[1]

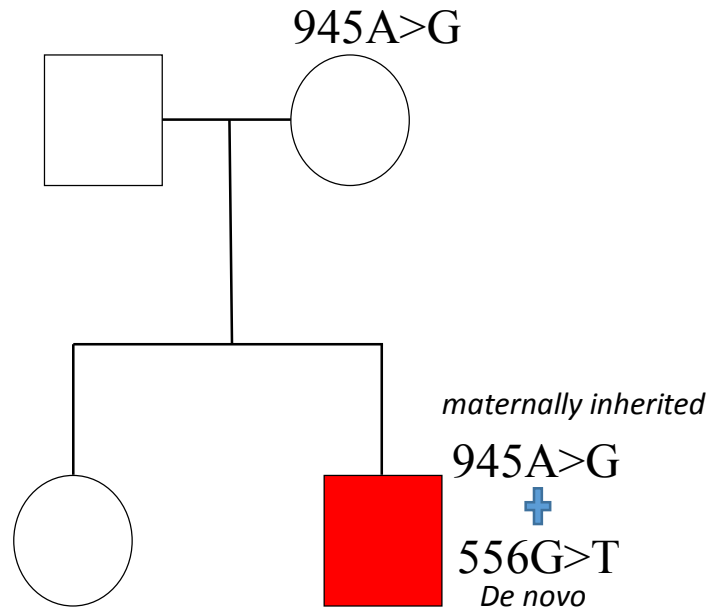
[1] Colleen Giblin Laboratories for Pediatric Neurology Research, Columbia University, New York, NY USA

[2] Clinical Cytogenetics Laboratory, Columbia University, New York, NY USA

[3] Department of Pediatrics, Columbia University, New York, NY USA

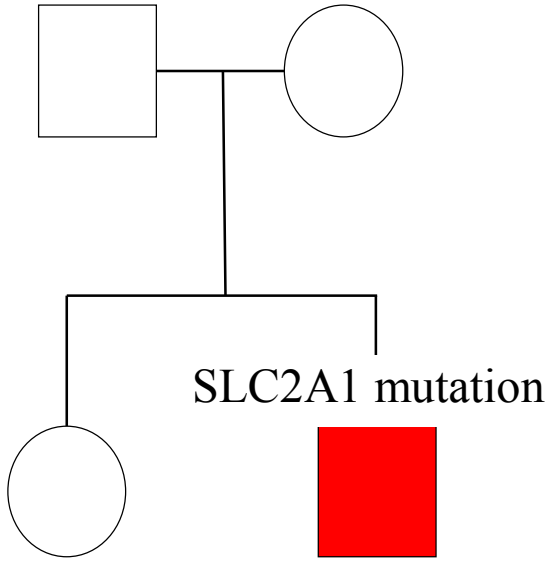
Abstract

Two families manifesting Glut1 deficiency syndrome (Glut1 DS) as an autosomal recessive trait are described. In one family, a severely affected boy inherited a mutated allele from his asymptomatic heterozygous mother. A *de novo* mutation developed in the paternal allele producing compound heterozygosity. In another family, two mildly affected sisters inherited mutations from their asymptomatic heterozygous consanguineous parents. RBC glucose uptake residual activity, a surrogate of haploinsufficiency, correlated with the clinical severity. These cases demonstrate that Glut1 DS may present as an autosomal recessive trait. The clinical pattern of inheritance is determined by the relative pathogenicity of the mutation and the resulting degree of haploinsufficiency.



Inheritance

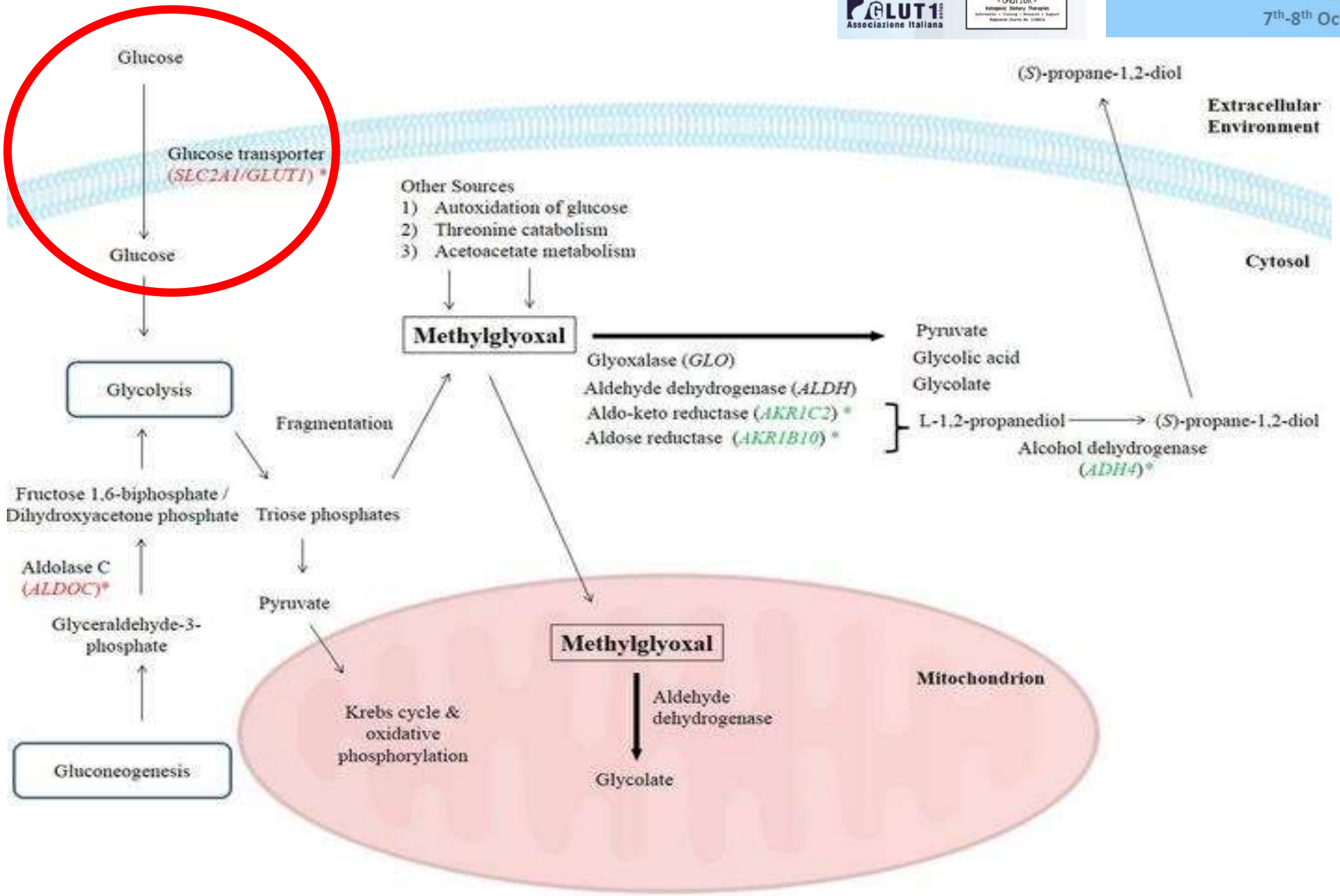
GLUT1-DS often presents as a sporadic disease with ‘de novo’ mutations producing haploinsufficiency and conferring symptomatic heterozygosity

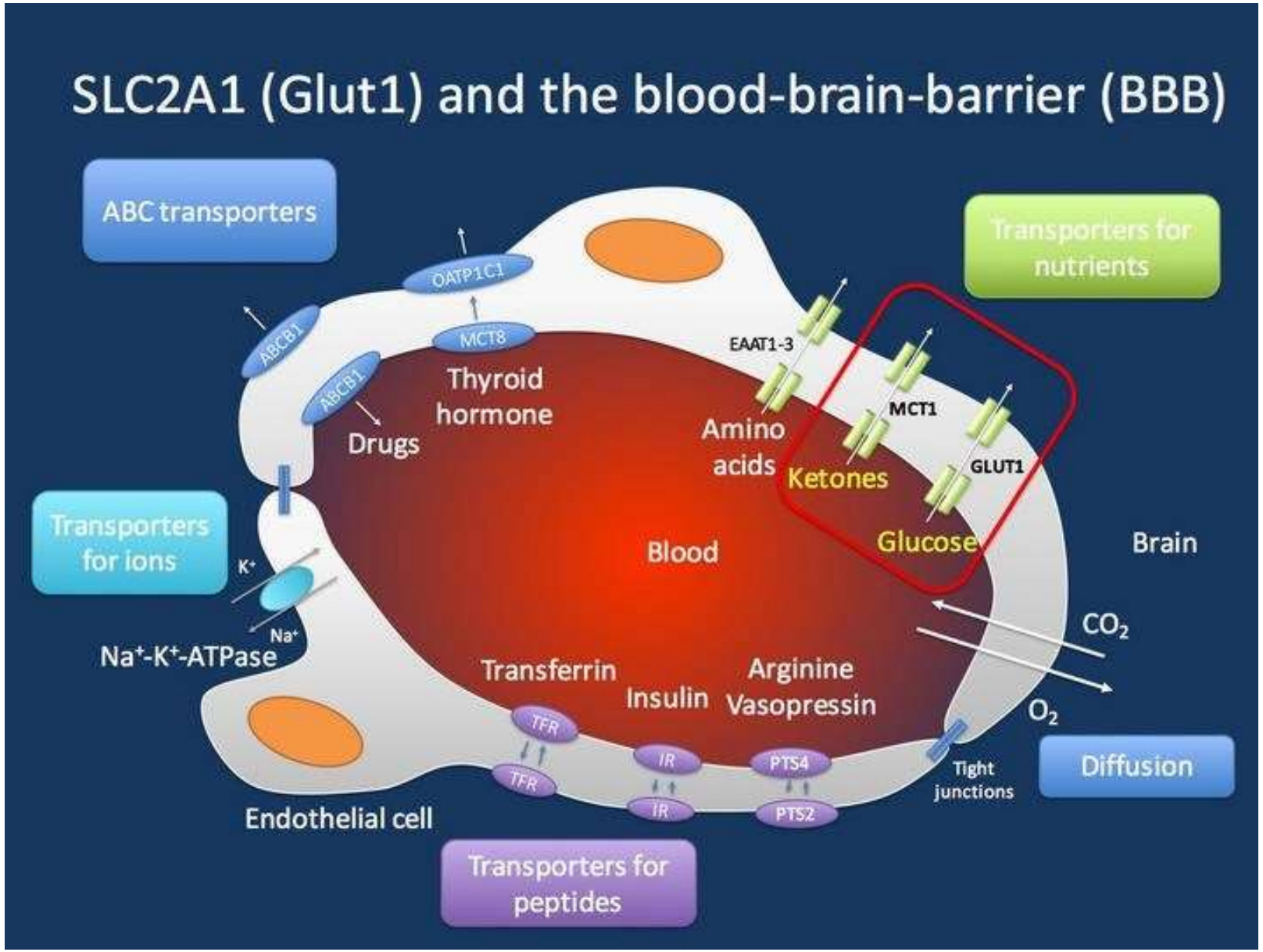


Genotype

- Several hot spots for recurrent mutations have been identified
  - Asn34
  - Gly91
  - Ser113
  - Arg126
  - Arg153
  - Arg264
  - Thr295
  - Arg333

- **All mutations either lead to absence or loss of function of one of the SLC2A1 alleles**


Glucose
Glucose transporter
(SLC2A1/GLUT1) *
Glucose
(S)-propane-1,2-diol
Extracellular Environment
Cytosol
Other Sources
1) Autoxidation of glucose
2) Threonine catabolism
3) Acetoacetate metabolism
Methylglyoxal
Glyoxalase (GLO)
Aldehyde dehydrogenase (ALDH)
Aldo-keto reductase (AKR1C2) *
Aldose reductase (AKR1B10) *
Pyruvate
Glycolic acid
Glycolate
L-1,2-propanediol
(S)-propane-1,2-diol
Alcohol dehydrogenase
(ADH4)*
Glycolysis
Fragmentation
Fructose 1,6-biphosphate /
Dihydroxyacetone phosphate
Triose phosphates
Aldolase C
(ALDOC)*
Glyceraldehyde-3-phosphate
Pyruvate
Methylglyoxal
Mitochondrion
Krebs cycle & oxidative phosphorylation
Aldehyde dehydrogenase
Glycolate
Gluconeogenesis


SLC2A1 (Glut1) and the blood-brain-barrier (BBB)
ABC transporters
Transporters for nutrients
Transporters for ions
Transporters for peptides
OATP1C1
ABCB1
ABCB1
MCT8
Thyroid hormone
Drugs
EAAT1-3
Amino acids
MCT1
Ketones
GLUT1
Glucose
Blood
Brain
K+
Na+
Na+-K+-ATPase
Transferrin
Insulin
Arginine Vasopressin
CO2
O2
TFR
TFR
IR
IR
PTS4
PTS2
Tight junctions
Diffusion
Endothelial cell

1st European Conference on Glut1 Deficiency

Milan, Italy

7th-8th October 2016

Rare disorder?

2011: close to 200 patients identified worldwide
2016: thousand/s patients..

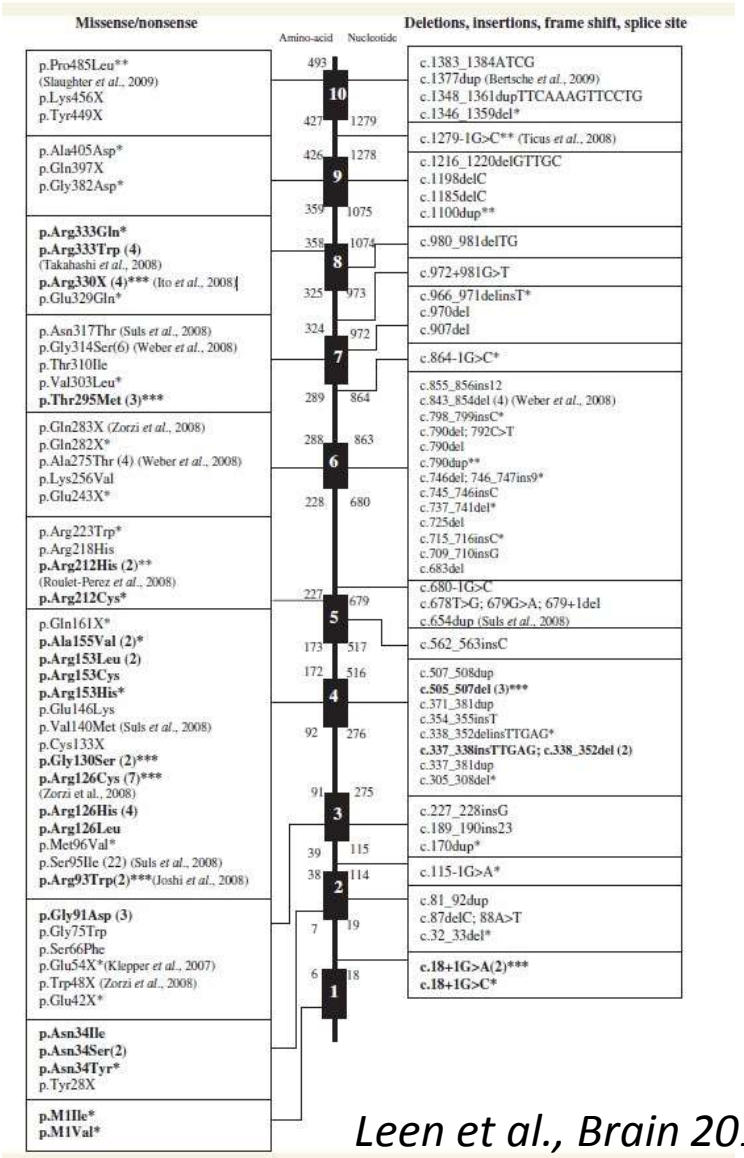
2004 to 2008

132 requests to analyse SLC2A1 gene

54 mutations (41%) (French study)

6/207= 2.8% - Danish study
Estimated frequency 1:83,000

Larsen et al., Epilepsia 2015



Leen et al., Brain 2010

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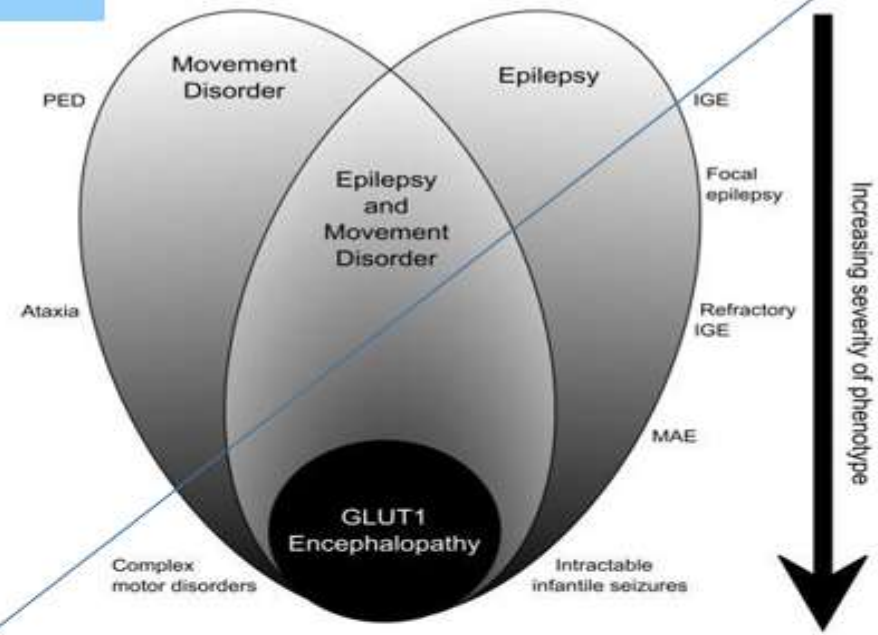
2016



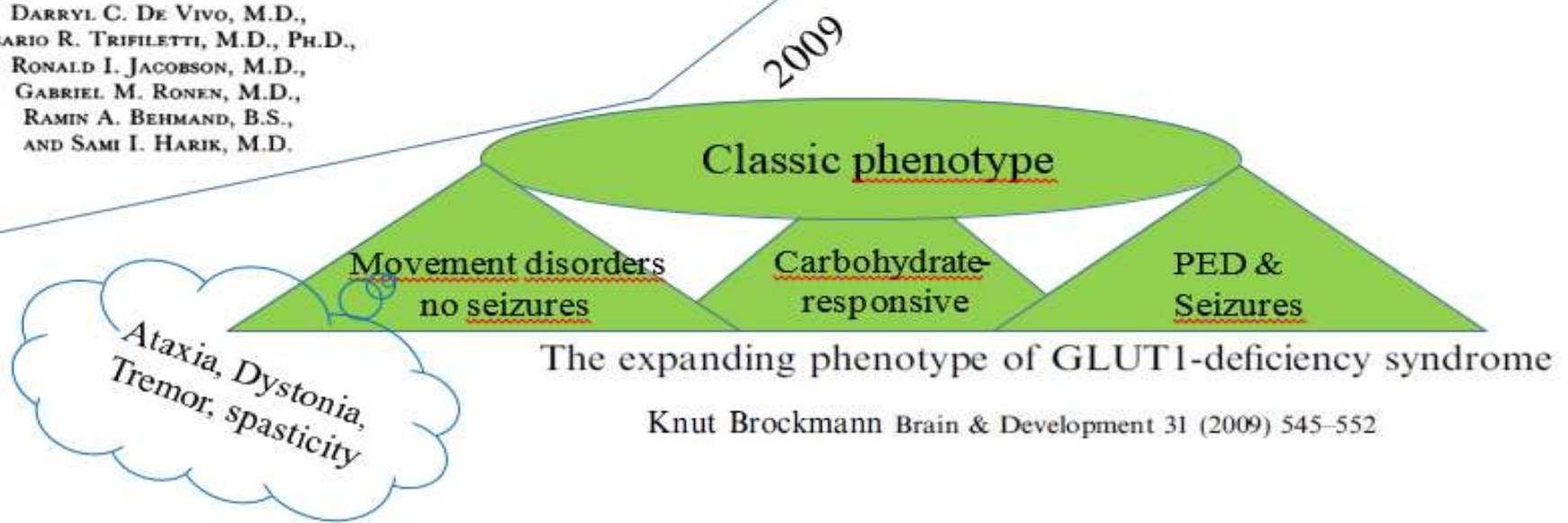
N Engl J Med
1991;325(10):703-9.

DEFECTIVE GLUCOSE TRANSPORT ACROSS THE BLOOD–BRAIN BARRIER AS A CAUSE OF PERSISTENT HYPOGLYCORRHACHIA, SEIZURES, AND DEVELOPMENTAL DELAY

DARRYL C. DE VIVO, M.D.,
ROSARIO R. TRIFILETTI, M.D., PH.D.,
RONALD I. JACOBSON, M.D.,
GABRIEL M. RONEN, M.D.,
RAMIN A. BEHMAND, B.S.,
AND SAMI I. HARIK, M.D.

1991

2009



The expanding phenotype of GLUT1-deficiency syndrome

Knut Brockmann Brain & Development 31 (2009) 545–552

Classic – De Vivo – GLUT1-DS phenotype

DEFECTIVE GLUCOSE TRANSPORT ACROSS THE BLOOD–BRAIN BARRIER AS A CAUSE OF PERSISTENT HYPOGLYCORRHACHIA, SEIZURES, AND DEVELOPMENTAL DELAY

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N Engl J Med 1991;325:703–9.

Table 1. Blood and Cerebrospinal Fluid Values for Glucose and Lactate in Two Children with Defective Glucose Transport across the Blood–Brain Barrier.*

Age	Blood Glucose	Blood Lactate	CSF Glucose	CSF Lactate†	CSF Glucose:Blood Glucose‡
mo		*millimoles per liter*			
Patient 1					
2.5	5.5	—	1.6	—	0.29
4.5	4.7	—	0.88	0.41	0.19
6.0	4.8	—	1.06	—	0.22
7.5	5.8	1.3	1.89	0.31	0.33
Patient 2					
5.5	—	—	1.4	1.0	—
5.8	6.7	—	1.6	1.3	0.24
6.0	4.6	1.0	1.5	0.9	0.33
9.0	9.4§	2.4	1.8	1.5	0.19
17.5	5.2	1.3	1.8	1.2	0.35
27.8	5.5	1.4	1.7	1.3	0.31

- Infantile onset seizures
- Delayed development
- Deceleration of head growth
- Paroxysmal events of non-epileptic nature
- Hypoglycorrhachia and reduced CNS/ blood glucose ratio
- Dramatic response to KD

SLC2A1 mutations and epilepsy type/syndromes

1st European Conference on Glut1 Deficiency
Milan, Italy
7th-8th October 2016

Early-Onset Absence Epilepsy Caused by Mutations in the Glucose Transporter GLUT1

Arvid Suls, MSc,[1–3] Saul A. Mullen, MBBS,[4] Yvonne G. Weber, MD,[5] Kristien Verhaert, MD,[6] Berten Ceulemans, PhD, MD,[3,6,7] Renzo Guerrini, MD,[8] Thomas V. Wuttke, MD,[5,9] Alberto Salvo-Vargas,[5,9] Liesbet Deprez, PhD,[1–3] Lieve R. F. Claes, PhD,[1–3] Albena Jordanova, PhD,[1–3] Samuel F. Berkovic, MD, FRS,[4] Holger Lerche, MD,[5,9] Peter De Jonghe, PhD, MD,[1–3,5] and Ingrid E. Scheffer, PhD, MBBS[4,10]

Ann Neurol. 2009 Sep;66(3):415-9

12% (4/34) early-onset absence epilepsy of patients

- 2 mutations de novo
- 2 mutations familial

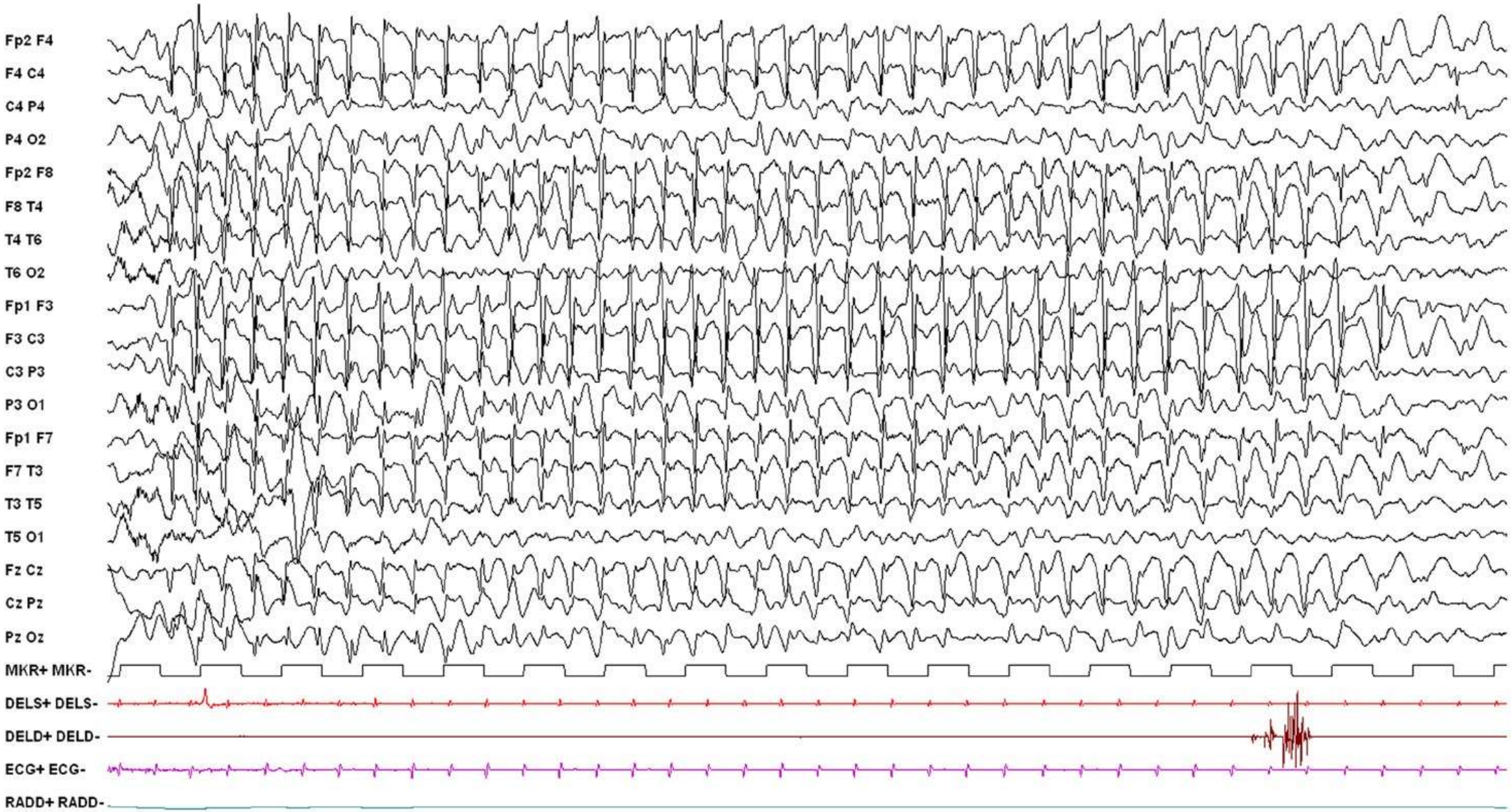
Glucose Transporter 1 Deficiency as a Treatable Cause of Myoclonic Astatic Epilepsy

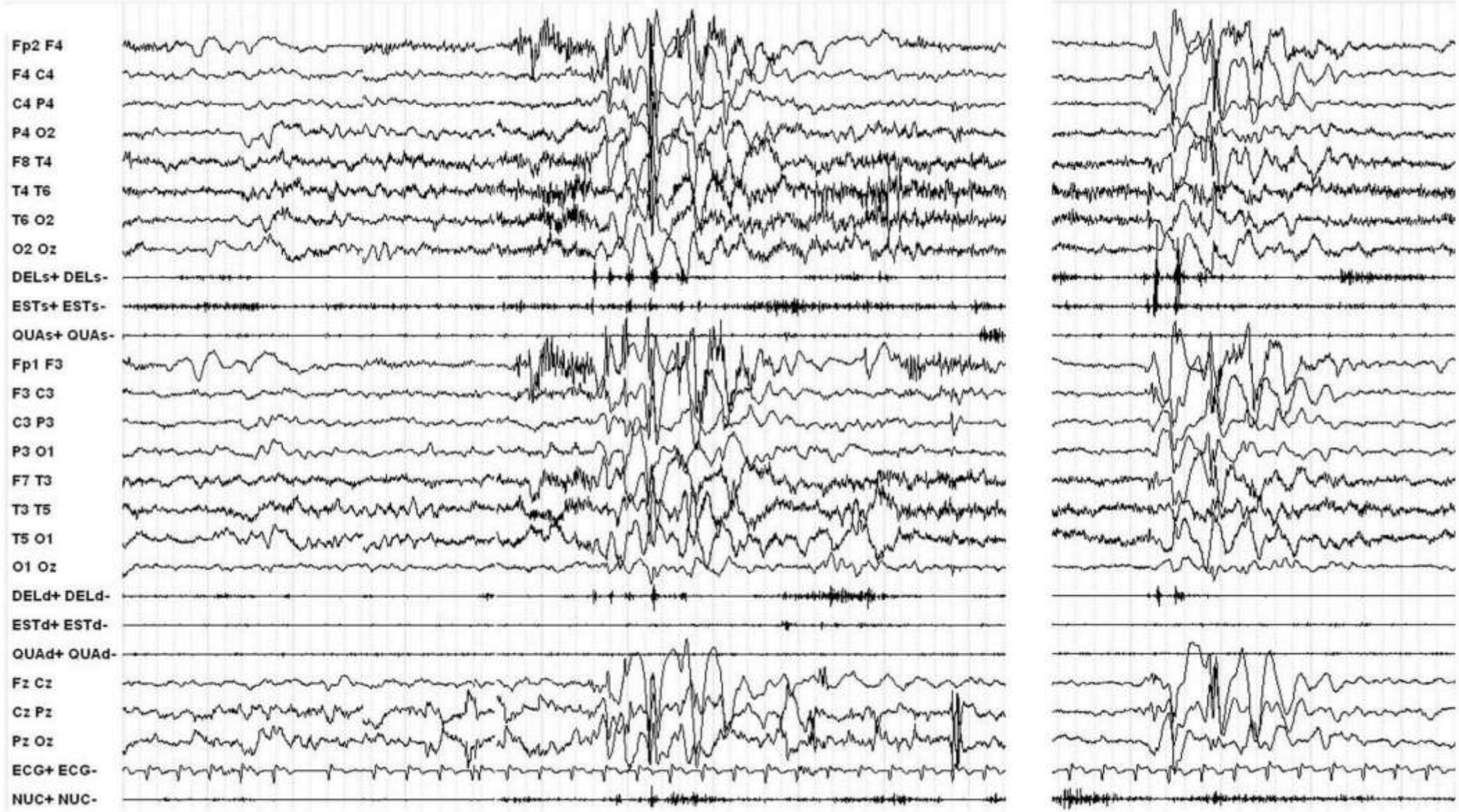
ARCH NEUROL/VOL 68 (NO. 9), SEP 2011

Saul A. Mullen, MBBS; Carla Marini, MD, PhD; Arvid Suls, PhD; Davide Mei, BSc; Elvio Della Giustina, MD; Daniela Buti, MD; Todor Arsov, MD, PhD; John Damiano, BSc; Kate Lawrence, BSc; Peter De Jonghe, MD, PhD; Samuel F. Berkovic, MBBS, MD; Ingrid E. Scheffer, MBBS, PhD; Renzo Guerrini, MD

Table. Clinico-molecular Features of the Patients With MAE and GLUT1 Deficiency

Patient/ Sex/ Age, y[a]	Sz Onset Age/Type	EEG Findings	AEDs/Sz Outcome	KD/Age at Onset	Cognitive Features	Neurological Examination	Movement Disorder Onset Age/Type	CSF Glucose Level/ CSF to Blood Glucose Level Ratio	*SLC2A1* Mutation
1/M/15	<36 mo/ My, My-At	GSW, GPSW	VPA + ESM; 5 y sz-free	Yes/ 13.5 y	Normal early development, progressive regression; at 5 y WPSI FSIQ score, 78; 6 y WPSI FSIQ score, 63; 7 y WPSI FSIQ score, 59; 13 y WISC-R FSIQ score <40	Tremor, mild dysarthric speech	6 y/PED > lower limbs (>R); ↑exercise; facial grimaces; duration 30-180 min; monthly frequency	35 mg/dL/NA	c.997C>T; p.Arg333Trp; parents, NA
2/M/4	8 mo/ My; 12 mo/ My-At	2.5- to 3-Hz GSW	VPA 1 y; sz-free	Yes/ 2.5 y	Mild motor and speech delay at 2 y and 2 mo (Griffiths score at 2 y, 62 and at 8 mo, 66)	Ataxia, dysarthric speech, poor motor performances, deceleration of head growth	None	32 mg/dL/0.42	c.1199G>A; p.Arg400His; de novo
3/M/12	24 mo/ My-At, My	GSW	VPA + TPM; 4 y sz-free	No	Mild motor and speech delay, progressive regression to severe ID	Dysarthric speech, ataxia, poor motor performances	6-7 y/PED > lower limbs (>L); ↑exercise	37 mg/dL/0.42	c.997C>T; p.Arg333Trp; de novo
4/M/28	48 mo/ At, Ab, GTCS, non-CSE	GSW	VPA + LTG; 4 y sz-free	No	Mild ID	Normal	None	NA	c.971C>T; p.Ser324Leu; maternal


Fp2 F4
F4 C4
C4 P4
P4 O2
Fp2 F8
F8 T4
T4 T6
T6 O2
Fp1 F3
F3 C3
C3 P3
P3 O1
Fp1 F7
F7 T3
T3 T5
T5 O1
Fz Cz
Cz Pz
Pz Oz
MKR+ MKR-
DELS+ DELS-
DELD+ DELD-
ECG+ ECG-
RADD+ RADD-


Fp2 F4
F4 C4
C4 P4
P4 O2
F8 T4
T4 T6
T6 O2
O2 Oz
DELs+ DELs-
ESTs+ ESTs-
QUAs+ QUAs-
Fp1 F3
F3 C3
C3 P3
P3 O1
F7 T3
T3 T5
T5 O1
O1 Oz
DELd+ DELd-
ESTd+ ESTd-
QUAd+ QUAd-
Fz Cz
Cz Pz
Pz Oz
ECG+ ECG-
NUC+ NUC-

The role of *SLC2A1* mutations in myoclonic astatic epilepsy and absence epilepsy, and the estimated frequency of GLUT1 deficiency syndrome

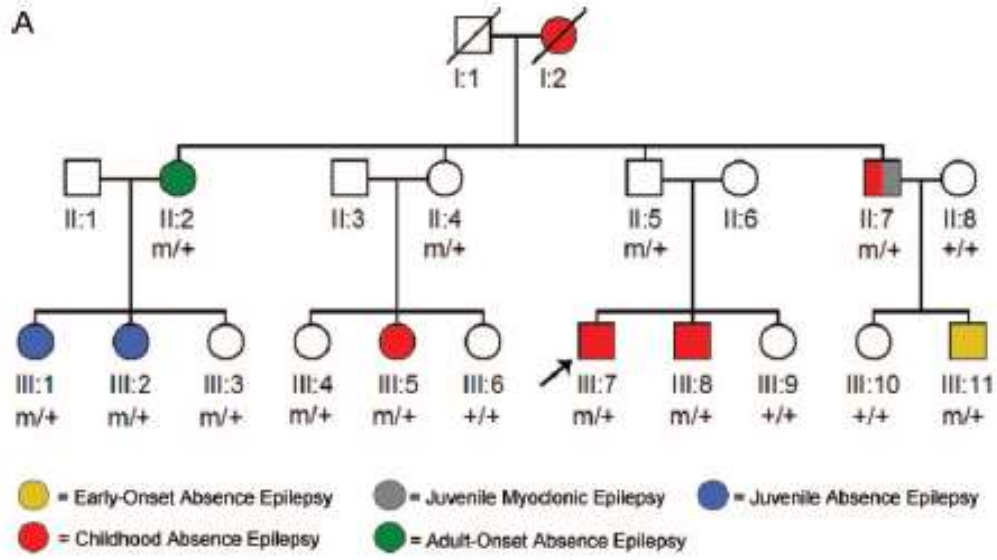
Larsen J, Johannesen KM, Ek J, Tang S, Marini C, Blichfeldt S, Kibaek M, von Spiczak S, Weckhuysen S, Frangu M, Neubauer BA, Uldall P, Striano P, Zara F; MAE working group of EuroEPINOMICS RES Consortium, Kleiss R, Simpson M, Muhle H, Nikanorova M, Jepsen B, Tommerup N, Stephani U, Guerrini R, Duno M, Hjalgrim H, Pal D, Helbig I, Møller RS.
Epilepsia. 2015;56(12):e203-8.

Mutations in SCL2A1 were detected in 5 (10%) of 50 patients with early onset absence epilepsy and in 1 (2.7%) of 37 patients with unselected epilepsies, ID and movement disorders

GLUT1 mutations are a rare cause of familial idiopathic generalized epilepsy

Striano P, Weber YG, Toliat MR, Schubert J, Leu C, Chaimana R, Baulac S, Guerrero R, LeGuern E, Lehesjoki AE, Polvi A, Robbiano A, Serratosa JM, Guerrini R, Nürnberg P, Sander T, Zara F, Lerche H, **Marini** C; EPICURE Consortium.

Neurology. 2012;78(8):557-62.



From splitting GLUT1 deficiency syndromes to overlapping phenotypes

Marie Hully [a,*], Sandrine Vuillaumier-Barrot [b], Christiane Le Bizec [b], Nathalie Boddaert [c], Anna Kaminska [d], Karine Lascelles [e], Pascale de Lonlay [f], Claude Cances [g], Vincent des Portes [h], Agathe Roubertie [i], Diane Doummar [j], Anne LeBihannic [k], Bertrand Degos [l], Anne de Saint Martin [m], Elisabeth Flori [n], Jean Michel Pedespan [o], Alice Goldenberg [p], Catherine Vanhulle [q], Soumeya Bekri [r], Anne Roubergue [j], Bénédicte Heron [j], Marie-Anne Cournelle [s], Alice Kuster [t], Alexis Chenouard [t], Marie-Noelle Loiseau [u], Vassili Valayannopoulos [f], Nicole Chemaly [a], Cyril Gitiaux [a], Nathalie Seta [b], Nadia Bahi-Buisson [a]

Epilepsy presentation at onset potentially predicts the outcome of patients with GLUT1-DS, since those with myoclonic seizures had a more ID, movement disorders and pyramidal signs than those with EOAE

53 point mutations and 5 deletions

87.5%= classic phenotype with

- intellectual deficiency (41.7%)
- epilepsy (75%)
- movement disorder (29%)

- initial symptoms at a medium age of 7.5 months

- 52%: myoclonic seizures

- 38%: Early Onset Absence Epilepsy

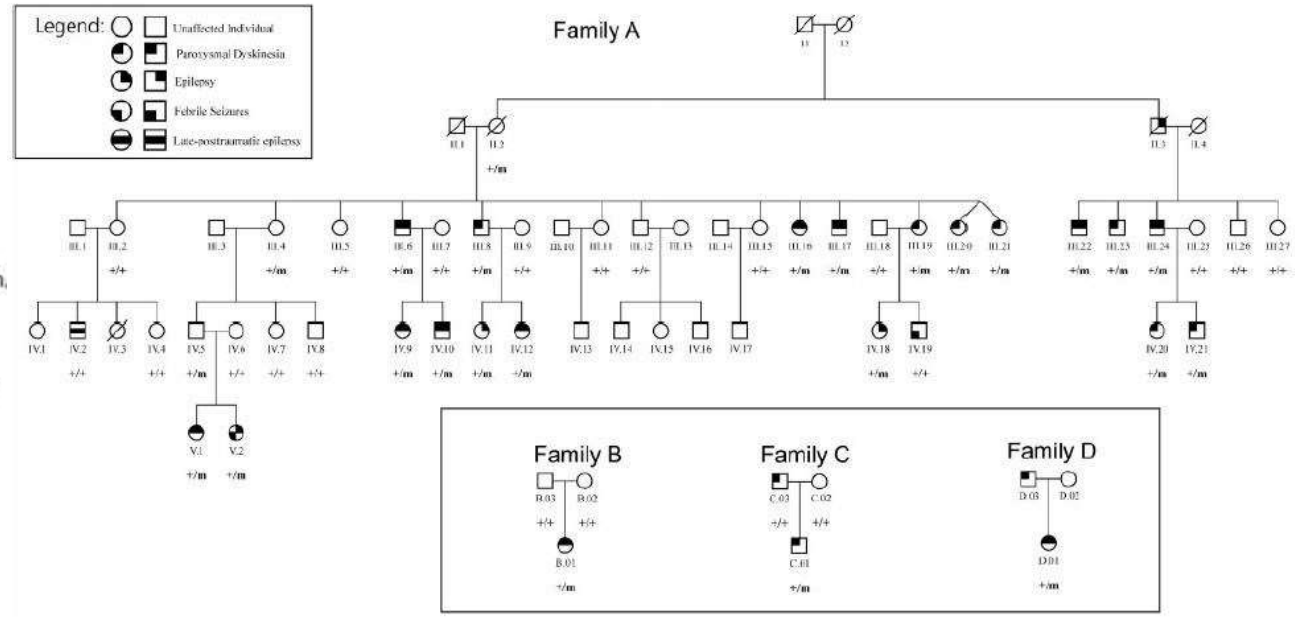
- Three patients evolved from a classic phenotype during early childhood to a movement disorder predominant phenotype at a late childhood/adulthood

European Journal of Medical Genetics 58 (2015) 443–454

Paroxysmal exercise-induced dyskinesia and epilepsy is due to mutations in *SLC2A1*, encoding the glucose transporter GLUT1

Brain (2008), **131**, 1831–1844

Arvid Suls,[1,2,3] Peter Dedeken,[4] Karolien Goffin,[5] Hilde Van Esch,[6] Patrick Dupont,[5] David Cassiman,[7] Judith Kempfle,[8,9] Thomas V. Wuttke,[8,9] Yvonne Weber,[8] Holger Lerche,[8,9] Zaid Afawi,[10] Wim Vandenberghe,[4] Amos D. Korczyn,[11] Samuel F. Berkovic,[12] Dana Ekstein,[13] Sara Kivity,[14] Philippe Ryvlin,[15] Lieve R. F. Claes,[1,2,3] Liesbet Deprez,[1,2,3] Snezana Maljevic,[8,9] Alberto Vargas,[8,9] Tine Van Dyck,[1,2,3] Dirk Goossens,[3,16] Jurgen Del-Favero,[3,16] Koen Van Laere,[5] Peter De Jonghe[1,2,3,17] and Wim Van Paesschen[4]



With intellectual disability and ataxia

J Inherit Metab Dis. 2003;26(6):559-63.

GLUT-1 deficiency without epilepsy--an exceptional case.

Overweg-Plandsoen WC[1], Groener JE, Wang D, Onkenhout W, Brouwer OF, Bakker HD, De Vivo DC.

Arch Neurol. 2009 Nov;66(11):1410-4. doi: 10.1001/archneurol.2009.236.

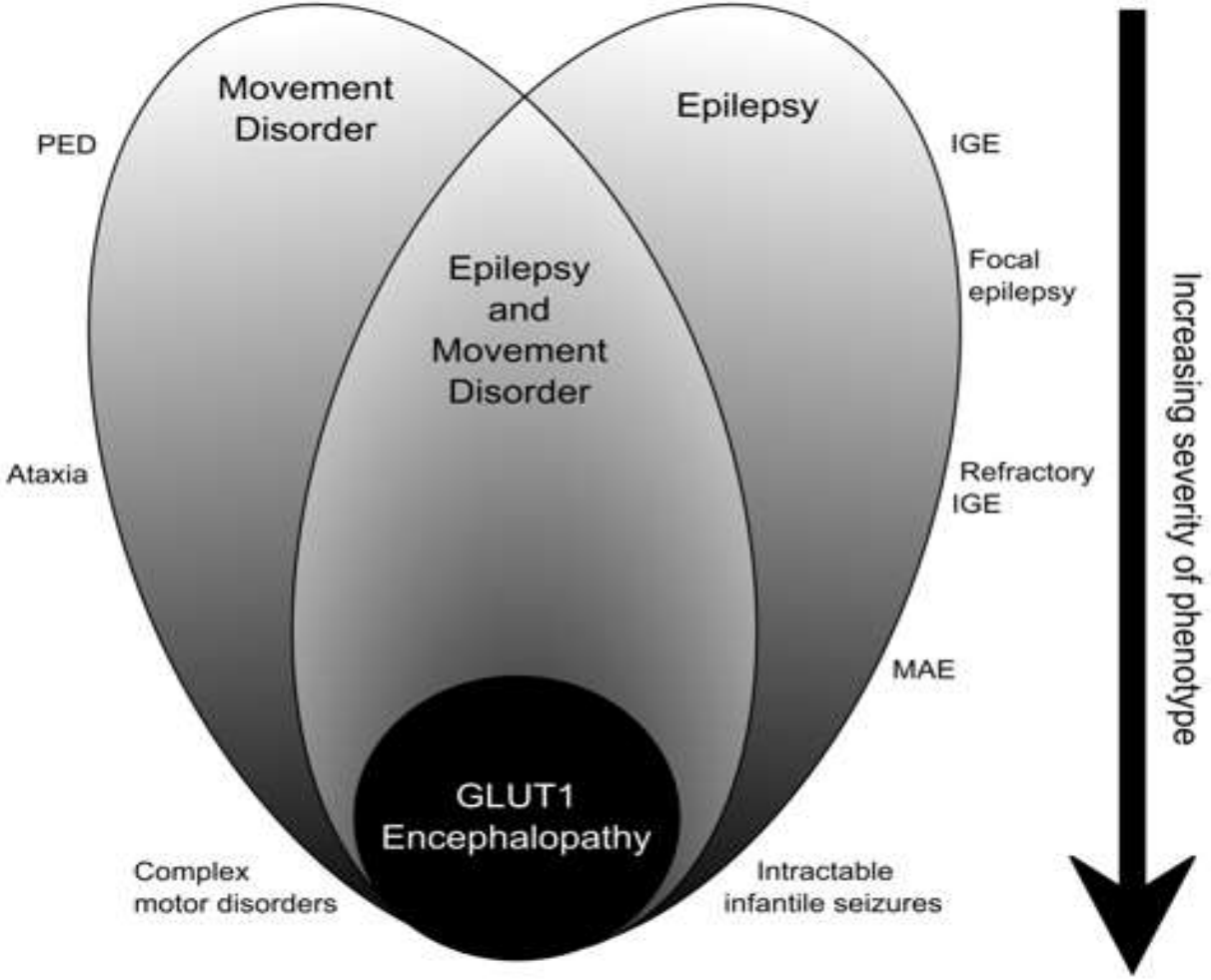
Childhood chorea with cerebral hypotrophy: a treatable GLUT1 energy failure syndrome.

Pérez-Dueñas B[1], Prior C, Ma Q, Fernández-Alvarez E, Setoain X, Artuch R, Pascual JM.

J Inherit Metab Dis. 2011 Apr;34(2):483-8. doi: 10.1007/s10545-010-9264-6. Epub 2011 Jan 13.

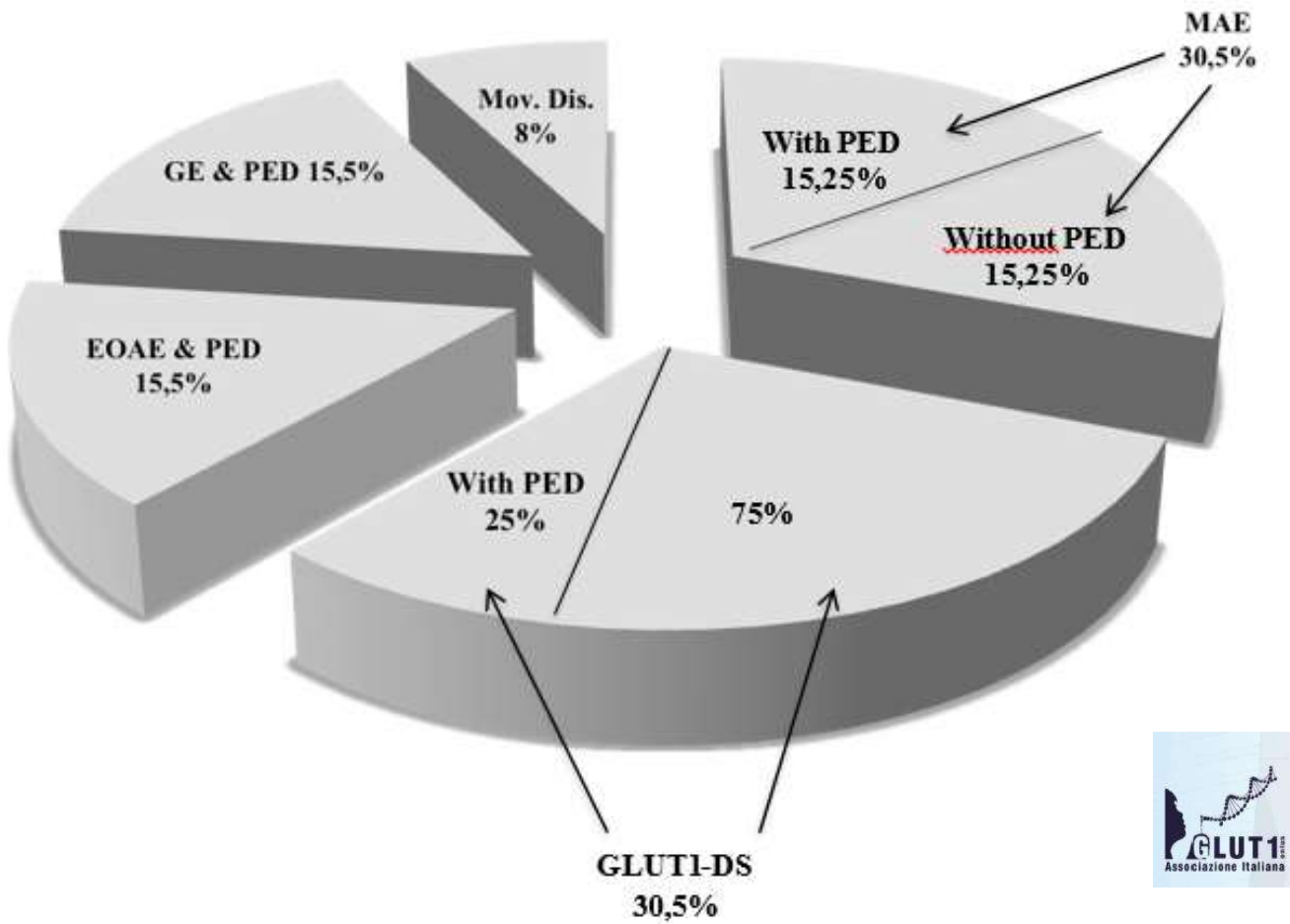
Dystonic tremor caused by mutation of the glucose transporter gene GLUT1.

Rouberque A[1], Apartis E, Mesnage V, Doummar D, Trocello JM, Roze E, Taieb G, De Villemeur TB, Vuillaumier-Barrot S, Vidailhet M, Levy R.



Mullen et al 2011

Graphic representation of the spectrum of disorders associated with *SLC2A1* gene mutations in our cohort (13 patients) *(unpublished data)*



1st European Conference on Glut1 Deficiency

Milan, Italy

7th-8th October 2016

Legend

EOAE: early onset absence epilepsy; **GE:** generalized epilepsy; **GLUT1-DS:** GLUT1 deficiency syndrome; **Mov. Dis:** movement disorder; **PED:** paroxysmal exercise induced dyskinesia

Genotype and phenotype correlations

1st European Conference on Glut1 Deficiency
Milan, Italy
7th-8th October 2016

- Type of mutation with the severity of intellectual disability (ID)

  Mild ID in
  - 79% of patients with missense mutations
  - 26% of patients with nonsense, frame shift, splice site or translation initiation mutation or multiple exon deletion
- Presence or movement disorders
  - 63% in patients with missense mutations
  - 88% with other type of mutations
- **100% multiple exon deletion have: early onset classical phenotyp**

Correlations

genotype	**CFS/blood glucose ratio**	**phenotype**
mutations with truncating effect	mean 0.30	early onset GLUT1DS
missense mutations	mean 0.40	late onset GLUT1-DS or non classical phenotype

From our cohort of patients carrying SLC2A1 mutations

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MUTATION	SYNDROME	GLYCORRHACHIA
MISSENSE	4 MAE 1 IGE 1 PED 1 DE VIVO	• mean: 36.5 mg/dl • median: 37 mg/dl • range: 31 mg/dl- 43 mg/dl
SPLICING	1 EOAE 1 DE VIVO	• mean: 35 mg/dl • median: 35 mg/dl • range: 32 mg/dl-38 mg/dl
TRUNCATING	1 DE VIVO	31 mg/dl
FRAME SHIFT	1 DE VIVO	29 mg/dl

- No Genotype-phenotype correlation
- Missense mutations: wide range of manifestations from early onset absence epilepsy to GLUT1-DS
- Mutations with truncating effect: GLUT1-DS
- 2 patients with same missense mutation and identical phenotype: myoclonic astatic epilepsy with childhood onset PED and adolescent onset behavioral disturbance

(unpublished data)

Conclusion

If GLUT1DS is suspected:

A Lumbar puncture first

B Genetic testing first

- ✓ Individual cases with normal glycorrhachia
- ✓ Relying on hypoglycorrhachia alone might be unhelpful for a definite diagnosis in individual cases- GLUT1 deficit might remain undiagnosed and untreated

Conclusion

- Genetic testing to confirm the clinical diagnosis
  - *SLC2A1* sequencing
  - *SLC2A1* MLPA (to detect deletions)
  - Parental analysis to explore inheritance
  - Genotype-phenotype correlation

- Mutations with truncating functional effect have a more severe phenotype