

CoQ₁₀ Deficiency Is Not a Common Finding in GLUT1 Deficiency Syndrome

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Abstract CoQ₁₀ deficiency has been recently described in tissues of a patient with GLUT1 deficiency syndrome. Here, we investigated patients and mice with GLUT1 deficiency in order to determine whether low CoQ is a recurrent biochemical feature of this disorder, to justify CoQ₁₀ supplementation as therapeutic option.

CoQ₁₀ levels were investigated in plasma, white blood cells, and skin fibroblasts of 16 patients and healthy controls and in the brain, cerebellum, liver, kidney, muscle, and plasma of 4-month-old GLUT1 mutant and control mice.

CoQ₁₀ levels in plasma did not show any difference compared with controls. Since most of the patients studied were on a ketogenic diet, which can alter CoQ₁₀ content in plasma, we also analyzed white blood cells and cultured skin fibroblasts. Again, we found no differences. In mice, we found slightly reduced CoQ in the cerebellum, likely an epiphenomenon, and activity of the mitochondrial respiratory chain enzymes was normal.

Our data from GLUT1 deficiency patients and from GLUT1 model mice fail to support CoQ₁₀ deficiency as a common finding in GLUT1 deficiency, suggesting that CoQ deficiency is not a direct biochemical consequence of defective glucose transport caused by molecular defects in the *SLC2A1* gene.

Introduction

GLUT1 deficiency syndrome (GLUT1DS) (OMIM 606777) is an autosomal dominant genetic trait due to mutations in the glucose transporter *SLC2A1* gene. GLUT1DS was first recognized in two children with seizures and persistently low glucose in the cerebrospinal fluid (CSF) (De Vivo et al. 1991; Seidner et al. 1998).

Since GLUT1 is the glucose transporter in the blood–brain barrier, the key hallmark is a low CSF glucose concentration in the presence of normoglycemia, with a CSF/blood glucose ratio commonly less than 0.4.

The clinical phenotype is variable; the most severe presentation is characterized by infantile epilepsy, developmental delay, cognitive impairment, spasticity, ataxia, and dystonia; some patients may present with paroxysmal head and eye movements, without epilepsy. A wide spectrum of mutations has been identified, but no obvious genotype–phenotype correlation has been detected (Pearson et al. 2013). The ketogenic diet is the standard of care, providing ketones in partial lieu of glucose as the alternative fuel for brain energy metabolism (Alter et al. 2015).

Recently, Yubero and colleagues reported a 15-year-old girl with GLUT1 deficiency and reduction of CoQ₁₀ levels in muscle, plasma, and skin fibroblasts (Yubero et al. 2014).

CoQ supplementation in the patient leads to a striking improvement of neurological phenotype, suggesting that

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adjunctive treatment with CoQ₁₀ may be wise when treating this disorder.

To determine if low levels of CoQ₁₀ are a common biochemical finding in GLUT1 deficiency, we measured CoQ levels in fibroblasts, plasma, and white blood cells of several patients with GLUT1 deficiency and in affected and unaffected tissues of a model mice with GLUT1 haploinsufficiency (GLUT1^{+/-}), as previously described (Wang et al. 2006).

Patients and Methods

Sixteen patients were diagnosed according to the following clinical and laboratory criteria: seizures, microcephaly, developmental delay, and low CSF/blood glucose ratio ($\sim 0.33 \pm 0.01$). Diagnosis was confirmed by molecular genetic analysis in 11. Informed consent was obtained from the parents of children who participated in this study. Plasma was collected from 14 patients; white blood cells were collected from eight patients, separated from plasma using Ficoll[®]-Paque, according to manufacturer instructions. Fibroblasts from five patients and six age-matched controls were cultured for CoQ₁₀ assessment as previously described (Lopez et al. 2006). Briefly, cells were grown in 15-cm-diameter culture plates until confluent. CoQ₁₀ was extracted in a hexane–ethanol mixture. The lipid component of the extract was separated by high-performance liquid chromatography (HPLC) on a reverse Symmetry[®] C18 3.5- μ m, 4.6 \times 150-mm column (Waters), using a mobile phase consisting of methanol, ethanol, 2-propanol, acetic acid (500:470:15:15), and 50 mM sodium acetate at a flow rate of 0.8 mL/min. The electrochemical detector consisted of an ESA Coulochem II with the following setting: guard cell (upstream of the injector) at +900 mV and conditioning cell at -650 mV (downstream of the column), followed by the analytical cell at +450 mV. CoQ₁₀ concentration was estimated by comparison of the peak area with those of standard solutions of known concentration. Four GLUT1^{+/-} heterozygous mice and four wild-type mice, aged 4 months, were euthanized; the brain, cerebellum, liver, kidney, muscle, and plasma were collected for biochemical analyses. CoQ₉ and CoQ₁₀ were extracted by mixing tissue extracts with 1-propanol, and measurement was performed as above. To measure activities of mitochondrial respiratory complexes, 40–70 mg tissue was homogenized in CPT medium (0.5 M Tris–HCl, 0.15 M KCl, pH 7.5) and centrifuged at 2,500g for 20 min at 4°C to obtain 10% homogenates. The supernatant was used for protein determination and enzymatic assays. Complex I and III (CI + III) activity was measured by observation of the reduction of cytochrome *c* (cyt *c*) at 550 nm. In brief, samples were incubated at 30°C in a

medium containing 100 mM KH₂PO₄ (pH 7.5), 10 mM KCN, 2 mM NADH, and 1 mM cyt *c*. The increase of absorbance was observed for 1 min and for an additional 2 min after addition of rotenone (10 μ g/mL). The residual activity in the presence of rotenone was subtracted from total activity. The results were expressed in nmol of reduced cyt *c*/min/g tissue. Complex II and III activity was measured by observation of the reduction of cytochrome *c* at 550 nm. Samples were incubated at 30°C in medium containing 100 mM KH₂PO₄ (pH 7.5), 30 mM succinate, 10 mM KCN, 2.5 μ M rotenone KCN, and 1 mM cyt *c*, and increase of absorbance was observed for 2 min. The results were expressed in nmol of reduced cyt *c*/min/g tissue. Citrate synthase (CS) activity was measured following the reduction of 1 mM 5,5'-dithiobis(2-nitrobenzoic acid) in 1 M Tris–HCl (pH 7.5) at 412 nm (30°C) in the presence of sample, 10 mM acetyl-CoA, and 10 mM oxalacetic acid. The results of CI + III, CII + III, and CIV were normalized to CS activity and to protein.

To determine the level of oxidative damage in the cerebellum, we performed Western blot analysis of carbonyl group content using the OxyBlot kit (Chemicon, Millipore Corp, Billerica, MA, USA) following the manufacturer's instructions. Briefly, 15 μ g of proteins were incubated with 2,4-dinitrophenylhydrazine to form 2,4-dinitrophenylhydrazine (DNP) derivatives. 2,4-Dinitrophenyl-derivatized proteins were separated on a 12% polyacrylamide gel and transferred to polyvinylidene difluoride membranes for 30 min at 50 V in Mini Trans-Blot cell apparatus (Bio-Rad, Hercules, CA, USA). The blots were hybridized with rabbit anti-DNP antibody and goat anti-rabbit horseradish peroxidase-conjugated secondary antibody (Chemicon, Millipore Corp, Billerica, MA, USA) and visualized by autoradiography using ECL substrate (Pierce, Rockford, IL, USA).

All animal studies were performed in accordance with protocols approved by the Columbia University Institutional Animal Care and Use Committee.

Mann–Whitney test was used to measure statistical significance ($p < 0.05$). Results are expressed as mean \pm standard deviation.

Results

At the time of sample collection, clinically all patients (4 males and 12 females, mean age 15.4 ± 7.2 years) were symptomatic, and all but two were on a ketogenic diet (Table 1).

CoQ₁₀ levels measured in plasma and white blood cells of seven patients showed no difference compared with controls (0.49 ± 0.06 vs. 0.41 ± 0.05 μ g/mL; and 62.53 ± 20.97 vs. 68.05 ± 22.3 μ g/mg prot, respectively) (Fig. 1a, b). We also assessed CoQ₁₀ levels in cultured skin

Table 1 Clinical features of GLUT1DS patients

Patient no.	Gender	Age of onset (month)	Age (year)	Disease duration (year)	Ketogenic diet	Clinical features	Genetic defect	Tissue studied
1	M	9	3	2	Y	S, M, D	Neg	P, WBC
2	F	12	4	3	Y	S, D	c.209 C>A	P, WBC
3	F	72	18	12	Y	S, D	c.438A>C	P, WBC
4	F	84	13	6	N	S, D	Neg	P
5	F	9	16	15	N	S, D	c.436G>A	P
6	M	19	30	28	Y	S, M, D	c.377G>A	P, WBC
7	F	4	20	20	Y	S, M, D	Microdeletion 3,336 bp (3,269 from intron 2 and the first 67 bp from exon 3)	P, WBC
8	F	3	19	19	Y	S, M, D	Microdeletion (start, 43119150; end, 43177300)	P, WBC
9	F	12	15	14	N	S, M	c.940G>A	P
10	M	3	2,5	2	Y	S, D	c.388G>C	P
11	F	30	14	12	N	S, M, D	c.276-1G>A	P
12	F	3	20	20	na	S, M, D	c.907delG	F
13	F	3	19	19	na	S, M, D	c.562_563insC	F
14	M	2	22	22	na	S, M, D	c.1383_1384insATCG	F
15	F	6	17	17	na	S, M, D	c.911_912insAC	F
16	F	1	14	14	na	S, M, D	c.791delG	F

P plasma, WBC white blood cells, F fibroblasts, na not available, S seizures, M microcephaly, D developmental delay

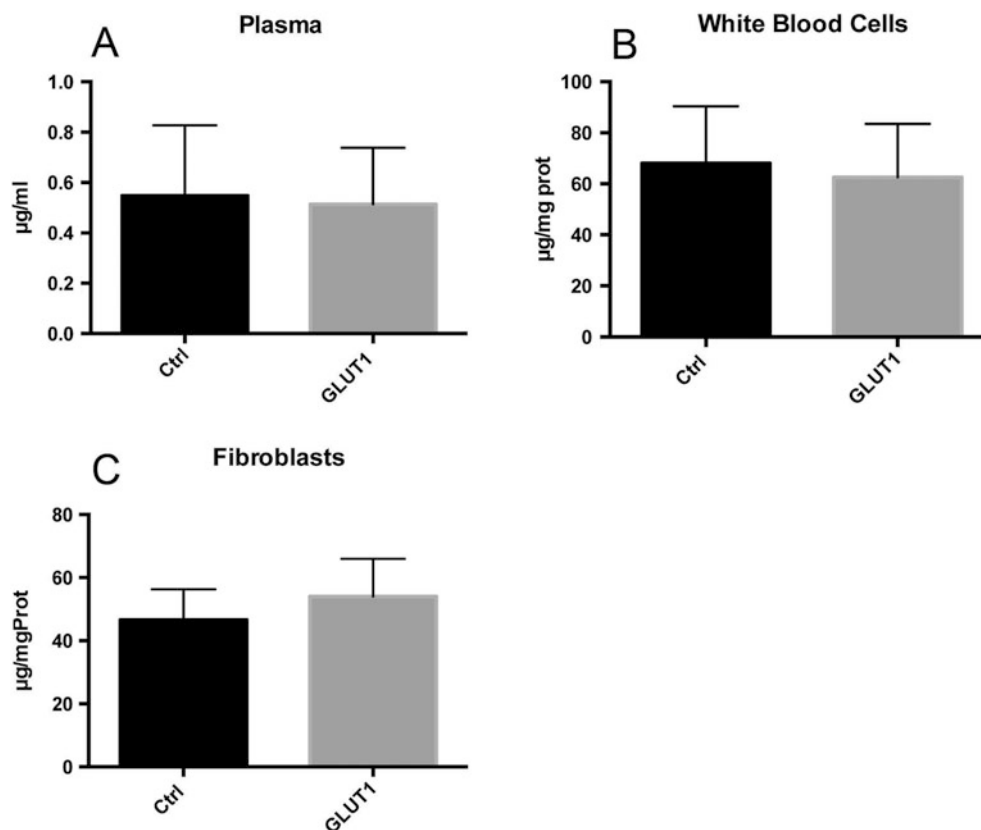


Fig. 1 CoQ₁₀ measured in (a) plasma of controls (ctrl; $n = 9$) and patients (GLUT1; $n = 8$), (b) white blood cells of controls (ctrl; $n = 6$) and patients (GLUT1; $n = 6$), and (c) skin fibroblasts of

controls (ctrl; $n = 6$) and patients (GLUT1; $n = 6$). Data are expressed as mean; bars represent standard deviation

fibroblasts from an additional six patients with GLUT1 deficiency and found no differences compared to 6 age-matched controls (54.05 ± 11.9 vs. 46.6 ± 9.7 $\mu\text{g}/\text{mg}$ prot) (Fig. 1c).

CoQ₁₀ deficiency has been shown to be tissue specific (Emmanuele et al. 2012). Thus, we measured CoQ levels in the brain, cerebellum, and skeletal muscle of GLUT1 mice. The liver and kidney were analyzed as non-affected tissues. CoQ₉, the most common ubiquinone in rodents, as well as CoQ₁₀ levels, was measured in wild-type and heterozygous animals. CoQ₉ was normal in the brain (22.4 ± 2.6 vs. 22 ± 2.8 ng/mg tissue) (Fig. 2b), muscle (14.7 ± 4 vs. 17 ± 7 ng/mg tissue) (Fig. 2c), kidney (136.3 ± 3 vs. 136 ± 3 ng/mg tissue), and liver (85.7 ± 27.7 vs. 84 ± 26.5 ng/mg tissue). A very mild although statistically significant reduction of CoQ₉ and CoQ₁₀ was present in the cerebellum (24.45 ± 0.6 vs. 23.65 ± 0.3 ng/mg tissue; $p < 0.05$) (Fig. 2a).

We did not observe any difference between GLUT1^{+/-} and wild-type animals when CoQ levels were normalized to CS, an index of mitochondrial mass (data not shown).

CoQ transports electrons from NADH dehydrogenase (complex I) and succinate dehydrogenase (complex II) to

cytochrome c reductase (complex III). Therefore, to assess the functional impact of CoQ levels on the mitochondrial electron transport, we measured the activity of complexes I + III and II + III in the cerebellum, normalized to proteins and CS, and we did not find any difference between GLUT1^{+/-} and wild-type animals (data not shown). Quantitative analysis of the blots carried out using the ImageJ software did not show any difference in protein oxidation between mutant and control mouse cerebellums (77.09 ± 19.05 vs. 68.22 ± 15.53).

Discussion

GLUT1DS is a rare neurological disorder with about 400 patients now reported in the literature worldwide (Pearson et al. 2013). The treatment with a ketogenic diet is based on the fact that brain metabolic fuels are limited to glucose and ketones. Recently, Yubero and colleagues reported a patient with GLUT1 deficiency, who showed consistently increased levels of blood lactate and alanine (Yubero et al. 2014). Mitochondrial function was investigated, and CoQ₁₀ was reduced in skeletal muscle, fibroblasts, and plasma.

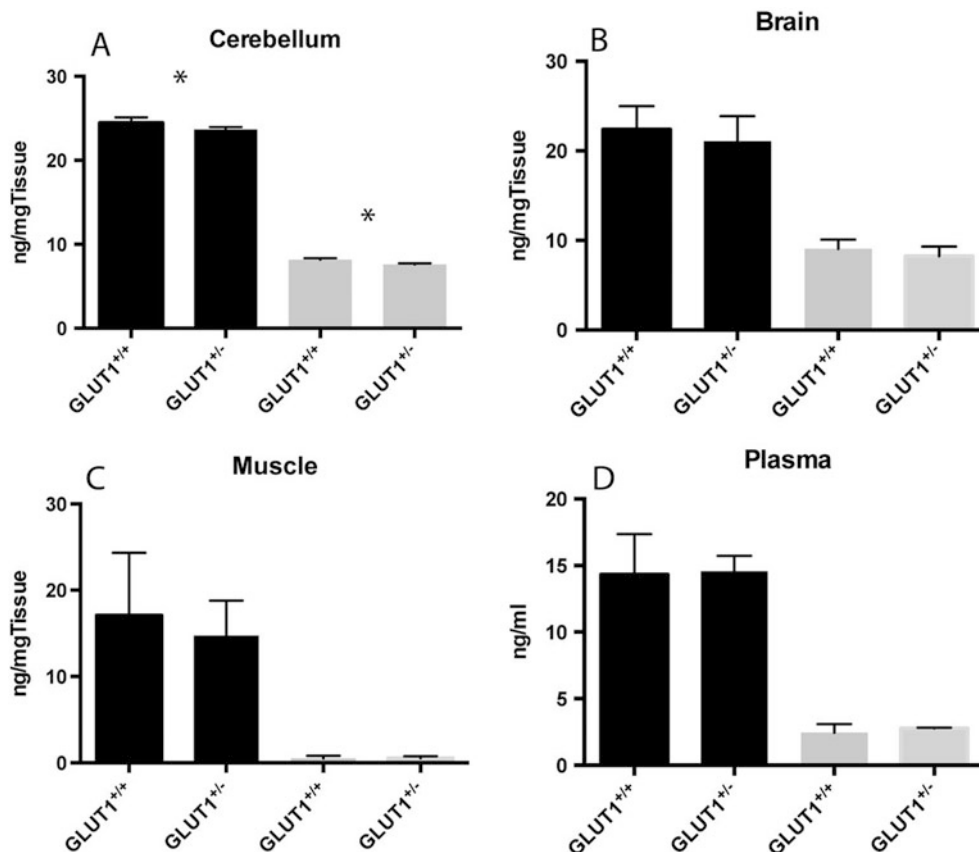


Fig. 2 CoQ₉ (solid black) and CoQ₁₀ (solid gray) in mutant (GLUT1^{+/-}; $n = 4$) and control (GLUT1^{+/+}; $n = 4$) mice: (a) cerebellum, (b) brain, (c) muscle, (d) plasma. Data are expressed as mean; error bars represent standard deviation. * $p < 0.05$

Supplementation therapy with CoQ₁₀ dramatically ameliorated some of the neurologic symptoms, especially cerebellar ataxia. Interestingly, CoQ₁₀ deficiency has been observed in different conditions associated with cerebellar ataxia suggesting a specific susceptibility of this brain region to oxidative stress and/or mitochondrial bioenergetics impairment due to CoQ₁₀ reduction (Emmanuele et al. 2012). GLUT1DS also is characterized clinically as an infantile-onset cerebellar phenotype, and the cerebellar region is unduly vulnerable as documented by brain imaging (Akman et al. 2015).

In order to assess if CoQ₁₀ reduction is a common feature in GLUT1DS, we collected tissue samples from 16 patients with GLUT1 deficiency. Plasma CoQ₁₀ levels, in seven patients, were comparable to controls. However, since plasma CoQ₁₀ levels can be influenced by diet (Emmanuele et al. 2012), we extended the studies to white blood cells (WBC) collected from peripheral blood. Again, the CoQ₁₀ levels in WBC were not different from controls, congruent with the plasma data. We also studied 6 skin fibroblast cell lines derived from GLUT1 patients and found normal levels of CoQ₁₀.

As is often the case in mitochondrial diseases, including CoQ deficiency, plasma and skin fibroblast CoQ levels do not necessarily reflect the biochemical phenotype (Emmanuele et al. 2012). Therefore, we decided to study affected and unaffected organs in model mice with GLUT1 deficiency. In GLUT1^{+/-} mice, CoQ levels were mildly reduced only in the cerebellum (~7%), while CoQ levels elsewhere in the brain, muscle, kidney, and liver were normal. The cerebellum, and other specific brain regions, is noticeably vulnerable to GLUT1 deficiency (Akman et al. 2015). Therefore, we think the slightly reduced CoQ levels in this brain region reflect the tissue pathology as an epiphenomenon. Reduced brain lipid synthesis has been shown in a different GLUT1 mouse model (Marin-Valencia et al. 2012) and also may account for reduced CoQ levels in the cerebellum. However, such mild deficiency can hardly alter mitochondrial energy metabolism, as confirmed by our observations of normal transport of electrons from complexes I and II to complex III.

Since CoQ₁₀ is the predominant antioxidant of the inner mitochondrial membrane, we studied oxidative stress as a possible consequence of CoQ₁₀ deficiency, and we did not find any evidence of oxidative stress in GLUT1^{+/-} mouse cerebellum, compared with controls.

In conclusion, our aggregate data from patients and model mice fail to support CoQ₁₀ deficiency as a common finding in GLUT1 deficiency, and CoQ deficiency cannot be viewed as a direct biochemical consequence of defective glucose transport associated with a disease-causing mutation in the *SLC2A1* gene. It is possible that the patient

described by Yubero et al. had the double misfortune of inheriting GLUT1 deficiency and a second (yet undefined) genetic disorder causing CoQ₁₀ deficiency.

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Synopsis

CoQ is not a recurrent biochemical feature of GLUT1DS; therefore, routine CoQ₁₀ supplementation as adjuvant therapy with the ketogenic diet cannot be justified.

Compliance with Ethics Guidelines

Conflict of Interest

Emanuele Barca declares that he has no conflicts of interest.

Maoxue Tang declares that he has no conflicts of interest.

Kristin Engelstad declares that he has no conflicts of interest.

Giulio Kleiner declares that he has no conflicts of interest.

Salvatore DiMauro declares that he has no conflicts of interest.

Catarina M. Quinzii declares that he has no conflicts of interest.

Darryl C. De Vivo declares that he has no conflicts of interest.

Informed Consent

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000. Informed consent was obtained from all patients for being included in the study.

Animal Rights

All institutional and national guidelines for the care and use of laboratory animals were followed.

Author Contribution

EB performed experiments and statistical analysis and wrote the manuscript, MT provided mouse samples and revised the manuscript, KE provided patients' data and revised the manuscript, GK performed experiments and wrote the manuscript, SD conceived and designed the project and revised the manuscript, CMQ conceived and designed the project and wrote the manuscript, and DCD diagnosed and takes care of the patients, conceived and designed the project, and wrote the manuscript.

References

- Akman CI, Provenzano F, Wang D, Engelstad K, Hinton V, Yu J, Tikofsky R, Ichese M, De Vivo DC (2015) Topography of brain glucose hypometabolism and epileptic network in glucose transporter 1 deficiency. *Epilepsy Res* 110:206–215
- Alter AS, Engelstad K, Hinton VJ, Montes J, Pearson TS, Akman CI, De Vivo DC (2015) Long-term clinical course of Glut1 deficiency syndrome. *J Child Neurol* 30(2):160–169
- De Vivo DC, Trifiletti RR, Jacobson RI, Ronen GM, Behmand RA, Harik SI (1991) Defective glucose transport across the blood–brain barrier as a cause of persistent hypoglycorrhachia, seizures, and developmental delay. *N Engl J Med* 325(10):703–709
- Emmanuele V, Lopez LC, Berardo A, Naini A, Tadesse S, Wen B, D'Agostino E, Solomon M, DiMauro S, Quinzii C, Hirano M (2012) Heterogeneity of coenzyme Q10 deficiency: patient study and literature review. *Arch Neurol* 69(8):978–983
- Lopez LC, Schuelke M, Quinzii CM, Kanki T, Rodenburg RJ, Naini A, DiMauro S, Hirano M (2006) Leigh syndrome with nephropathy and CoQ10 deficiency due to decaprenyl diphosphate synthase subunit 2 (PDSS2) mutations. *Am J Hum Genet* 79(6):1125–1129
- Marin-Valencia I, Good LB, Ma Q, Duarte J, Bottiglieri T, Sinton CM, Heilig CW, Pascual JM (2012) Glut1 deficiency (G1D): epilepsy and metabolic dysfunction in a mouse model of the most common human phenotype. *Neurobiol Dis* 48(1):92–101
- Pearson TS, Akman C, Hinton VJ, Engelstad K, De Vivo DC (2013) Phenotypic spectrum of glucose transporter type 1 deficiency syndrome (Glut1 DS). *Curr Neurol Neurosci Rep* 13(4):342
- Seidner G, Alvarez MG, Yeh JI, O'Driscoll KR, Klepper J, Stump TS, Wang D, Spinner NB, Birnbaum MJ, De Vivo DC (1998) GLUT-1 deficiency syndrome caused by haploinsufficiency of the blood–brain barrier hexose carrier. *Nat Genet* 18(2):188–191
- Wang D, Pascual JM, Yang H, Engelstad K, Mao X, Cheng J, Yoo J, Noebels JL, De Vivo DC (2006) A mouse model for Glut-1 haploinsufficiency. *Hum Mol Genet* 15(7):1169–1179
- Yubero D, O'Callaghan M, Montero R, Ormazabal A, Armstrong J, Espinos C, Rodriguez MA, Jou C, Castejon E, Aracil MA, Cascajo MV, Gavilan A, Briones P, Jimenez-Mallebrera C, Pineda M, Navas P, Artuch R (2014) Association between coenzyme Q 10 and glucose transporter (GLUT1) deficiency. *BMC Pediatr* 14(1):284