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Case Report

Usefulness of diagnostic tools in a GLUT1 deficiency syndrome patient with 2 inherited mutations

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Abstract

In some patients with GLUT1 deficiency syndrome (GLUT1-DS), the diagnosis can be difficult to reach. We report a child with 2 inherited mutations suggesting an autosomal recessive transmission of *SLC2A1* mutations.

Methods: The child and her parents were explored with erythrocyte 3-O-methyl-D-Glucose uptake, glucose uptake in oocytes expressing GLUT1 with the gene mutations and measure of the expression of GLUT1 at the surface of the circulating red blood cells by flow cytometry (METAglut1™ test).

Results: Both erythrocyte glucose uptake and glucose uptake in oocyte with the patient's mutations did not support the diagnosis of a mild GLUT1-DS phenotype with autosomal recessive transmission of *SLC2A1* mutations. Instead, GLUT-1 expression at the surface of the erythrocytes appeared to better correlate with the clinical phenotypes in this family.

Conclusion: The diagnostic value of these functional/expression tools need to be further studied with a focus on mild phenotype of GLUT1-DS.

Keywords: Diagnosis; Epilepsy; Ketogenic diet; GLUT1 deficiency syndrome

1. Introduction

Glucose transporter type 1 deficiency syndrome (GLUT1-DS) is a genetic-metabolic disorder. Most of the cases are caused by heterozygous mutations in the *SLC2A1* gene encoding the glucose transporter GLUT1, which allows the transport of glucose across the brain barrier. This results in the decrease of cerebral glucose which is the major substrate of brain energy. A prompt diagnosis is crucial to initiate a ketogenic diet allowing to provide ketones as an alternate energy substrate to glucose for the brain.

GLUT1-DS was first described as an infantile encephalopathy with early refractory epilepsy, delayed neurological development and microcephaly. In the recent years, the spectrum of GLUT1-DS has expanded with multiples phenotypes from mild to severe forms. In

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most cases, the disease appears in infancy or childhood, but onset in adulthood can occur with mild symptoms. There is no specific sign of GLUT1-DS on brain MRI or on the EEG recording[1]. The diagnosis is currently based on the clinical symptoms, the glycorrhachia compared to the glycemia and the molecular analysis of *SLC2A1* gene.

Clinical situations can be challenging. Consequently, molecular analysis of the *SLC2A1* gene is required to confirm the diagnosis, but could be sometimes inconclusive. 70–90% of the patients carry *SLC2A1* mutations [1]. Most mutations are autosomal dominant and acquired *de novo*, but some cases are described with an autosomal recessive mode of inheritance [2–5].

Other diagnostic tools have been developed but their diagnosis value remain to be established. The erythrocyte 3-O-methyl-D-Glucose (3-OMG) uptake is based on the principle that erythrocytes express high levels of the GLUT1 transporter. A cutoff point between patients and controls has been set at 74% of glucose uptake [5]. The use of oocytes to express patient mutations has been also developed to functionally assess the glucose uptake in an *in vitro* system [6,7]. More recently, a flow cytometry analysis of GLUT1 surface expression on circulating red blood cells (RBC) showed that GLUT1-DS patients typically exhibit at least 20% decreased expression of GLUT1 at the surface of RBC compared to controls [8].

Here, we report a family with a child that had two inherited *SLC2A1* mutations which has been explored with the three diagnostic tools described above.

2. Patients

The patient is a girl born from unrelated parents. Pregnancy and delivery were normal. The mother is healthy with an history of one episode of "abnormal movements" when she was 6 months-old without clear diagnosis. A seizure had been suspected at the time. The father has migraine without any aura. Before the age of 6 months, the patient was admitted after a first neurological episode interpreted as seizure. When she was 6-month-old, she experienced an other neurological attack consisting in divergence of eyes lasting for 15 min. Electroencephalogram and brain MRI were normal. The CSF analysis revealed an hypoglycorrhachia (CSF/blood: 1.9 mM/4.8 mM = 0.39; confirmed one week later: 1.7/4.1 = 0.41; CSF lactate: 0.9 mmol/l). We initiated a modified ketogenic diet when she was 7-month-old. She didn't experience any symptom until the age of 4 years, when she was admitted for an episode of left hemiparesis for a few hours associated with modified consciousness during the first hour. This occurred while the diet had been modified with increased carbohydrate intake from 30 to 35–45 g per day. Carbohydrate intake was then restricted to 30 g per day. The patient is now 7-year-old with normal psychomotor development and academic achievements. She is currently receiving 60 g of carbohydrates. She has no clinical symptom except for regular episodes of lower limb paroxysmal hypotonia after her dance class that might be related to her condition.

Ethical approval as well as the consents of both parents have been obtained. At the time of diagnosis, a molecular analysis of *SLC2A1* found two missense variants: c.653G>A,p.Arg218His inherited from her father and c.589G>C,p.Ala197Pro inherited from her mother. The father's variant has already been described in GLUT1-DS patients but predict mostly in silico tolerated [9,10]. The mother's variant has not yet been reported as pathogenic but predicts as being in silico. CSF glucose analyses were not possible in the parents. Glucose uptake was normal for the patient and both parents (Table 1). Fig. 1 reports the data from the glucose uptake from oocytes expressing the GLUT1 mutations was also normal in all 3 individuals (Fig. 1). Instead, the flow cytometry analysis of GLUT1 expression at the surface of RBCs (METAglut1™, METAFORA biosystems, France; technical description and norms are explained in details in [8] as well as in the supplementary material) recently showed a significantly decreased GLUT1 expression in the patient and her mother (Table 1). An informed consent was obtained following a full explanation of the procedures.

3. Discussion

The mild phenotype of our patient and the presence of two inherited *SLC2A1* mutations have led to diagnosis uncertainty for a while. We conducted two functional

Table 1
Results of different tests performed in the family.

	CSF/blood ratio	Molecular genetic *SLC2A1*	RBC glucose transport analysis	Flow cytometry analysis of GLUT1 surface expression on circulating RBC
Child	0.39	c.589G>C,p.Ala197Pro	78% of the control	54% of mean control
	0.41	c.653G>A,p.Arg218His		
Father	Not Done	c.653G>A,p.Arg218His	87% of the control	85% of mean control
Mother	Not Done	c.589G>C,p.Ala197Pro	81% of the control	65% of mean control

Thresholds interpretation: less than 74% residual uptake activity is considered positive, while less than 80% residual expression of GLUT1 on RBC is considered positive.

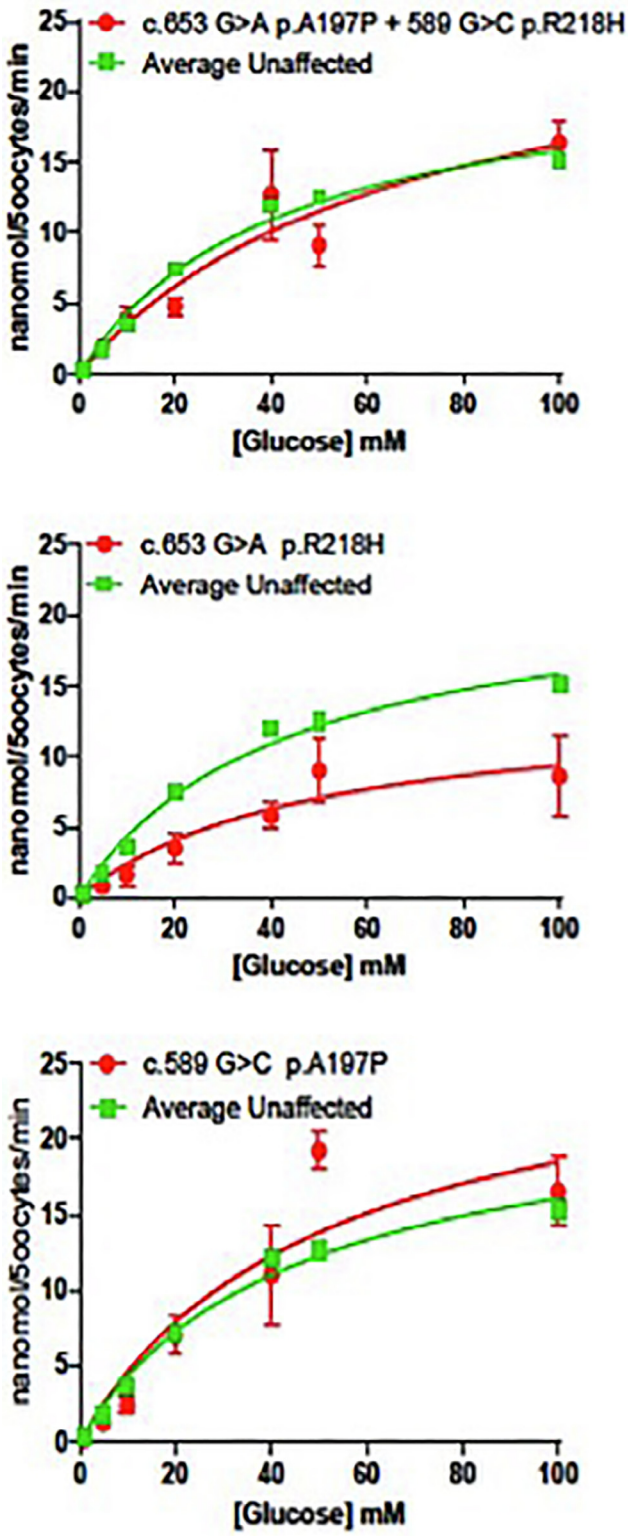

Fig. 1. Glucose uptake in oocytes expressing the GLUT1 mutations from the patients (upper part; green curve), the father (middle part; green curve) and the mother (lower part; green curve). The uptake over time is reported for each member of the family. The green curves report the data from the mutation found in the patients and the red curves are the controls. No significant differences in uptake were revealed. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

analyses that did not help us to reach a final diagnosis. Nonetheless, the clinical history had been consistent with a mild phenotype of GLUT1-DS. Furthermore, GLUT1 expression at the surface of the RBCs (METAglut1™) was similar to what has been reported in GLUT1-DS patients and may support the diagnosis in our patient.

The family of our patient has been complex for diagnosis reasoning. There are several reported families with two mutated alleles in *SLC2A1*, in which the parents are totally asymptomatic and have different mutations in *SLC2A1* gene [11,12]. This mode of inheritance is not exceptional in neurological disorders. Despite the description in other GLUT1-DS patients [9,10], the variant inherited from her father is reported 20 times in the Genome Aggregation Database (1:14,000 0.0072% in the general population and 0.11% in Ashkenazi Jewish) (http://gnomad.broadinstitute.org/) suggesting finally a likely incomplete penetrance of a mild variant as described for another one [13]. This correlates with the absence of any neurological symptom, except migraine, in the father, METAglut1™ test result and in silico prediction of tolerated variation. The mother has a predicted pathogenic variant not described to date, and the only suggestive symptom is a possible acute neurological attack in infancy. The p.Arg218His father's variant could have a likely modifying clinical effect as seen in Duarte variant Galactosemia when in trans configuration with a pathogenic allele [14].

Functional assay such as erythrocyte 3-O-methyl-D-Glucose (3-OMG) uptake has been developed as a diagnostic tool. In a study including 109 patients with hypoglycorrhachia, a correlation between the mean erythrocyte 3-OMG uptake and clinical severity has been reported after the exclusion of 35 patients with normal erythrocyte 3-OMG uptake. If the authors report a high sensitivity and a high specificity for abnormal low uptake value of 74% of the normal activity, the predictive value at the individual level remains to be established in particular in patients with mild phenotype. This test is an important functional test but not available on a clinical basis. The use of oocyte expressing *SLC2A1* mutations found in the patients avoid some limitations from the RBC analysis that requires fresh and metabolically functional red cells. This is a technical limitation when samples have to be transported over distance. However, the oocyte expression study used for the first time to explore concomitantly 2 mutations was not helpful either for the diagnosis of this family.

Recently, a proof of concept study has shown that GLUT1 expression level at the surface of RBC could be a cost effective and rapid test for GLUT1-DS in particular with regards to the diagnosis delays and the expending phenotypic presentations [6]. This study also evaluated the level of expression of GLUT1 on RBC and its variability in the general population. In our

patient and her parents, we found the following levels of expression: −45% in our patient (i.e. 31 percentile of the GLUT1-DS population and <1 percentile of the general population); −35% in her mother (i.e. 85 percentile of the GLUT1-DS population and <1 percentile of the general population) and −15% in her father (i.e. 96 percentile of the GLUT1-DS population and 8 percentile of the general population). In our patient with a mild phenotype, GLUT1 expression at the RBC surface provides interesting insights for the diagnosis to link to molecular data. The mother is likely to have a mild phenotype with limited expression during infancy while the father has a mild frequent variant with incomplete penetrance and the patient may have a likely more severe phenotype than her mother related to the presence of the father variant on the other allele. Further studies need to validate the predictive value of this test, in particular in patients with mild phenotype and inconclusive erythrocyte or oocyte glucose uptake assay. It would be also of interest to decipher the mechanisms of the defect of delivery of the GLUT1 protein at the RBC surface.

4. Conclusion

The data obtained here by 3 functional/expression analyses of GLUT1 is puzzling, and proves the complex interplay between mutations, protein expression/function, and symptoms. In this family, GLUT1 expression at the RBC surface appeared to be more sensitive than uptake assays conducted both on RBC and oocytes [8]. A possible explanation would be that the uptake assays are highly sensitive to pre-analytical conditions resulting into a lack of robustness for routine use. Conversely, RBC membrane architecture is highly stable when stored in proper conditions for days, making protein quantification more reliable. Overall, GLUT1 expression at the RBC surface could be a promising diagnostic tool. Likewise, functional assays and protein quantification of GLUT1 on RBC may be different than the expression and behavior of GLUT1 at the blood brain barrier.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.braindev.2019.05.008.

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