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Prospective Multicenter Validation of a Simple Blood Test for the Diagnosis of Glut1 Deficiency Syndrome

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Abstract

Background and Objective

GLUT1 deficiency syndrome (Glut1DS) is a treatable neurometabolic disease that causes a wide range of neurologic symptoms in children and adults. However, its diagnosis relies on an invasive test, that is, a lumbar puncture (LP) to measure glycorrhachia, and sometimes complex molecular analyses of the *SLC2A1* gene. This procedure limits the number of patients able to receive the standard of care. We wished to validate the diagnostic performance of METAglut1, a simple blood test that quantifies GLUT1 on the erythrocyte surface.

Methods

We performed a multicenter validation study in France, involving 33 centers. We studied 2 patient cohorts: a prospective cohort consisting of patients with a clinical suspicion of Glut1DS explored through the reference strategy, that is, LP and analyses of the *SLC2A1* gene, and a retrospective cohort that included patients previously diagnosed with Glut1DS. All patients were blind-tested with METAglut1.

Results

We analyzed 428 patients in the prospective cohort, including 15 patients newly diagnosed with Glut1DS, and 67 patients in the retrospective cohort. METAglut1 was 80% sensitive and >99% specific for the diagnosis of Glut1DS. Concordance analyses showed a substantial agreement between METAglut1 and glycorrhachia. In the prospective cohort, the positive predictive value of METAglut1 was slightly higher than that of glycorrhachia. METAglut1 succeeded to identify patients with Glut1DS with *SCL2A1* mosaicism and variants of unknown significance.

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Class of Evidence
Criteria for rating therapeutic and diagnostic studies
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Glossary

CAE = childhood absence epilepsy; **EAOE** = early absence onset epilepsy; **Glut1DS** = GLUT1 deficiency syndrome; **ID** = intellectual disability; **JAE** = juvenile absence epilepsy; **LP** = lumbar puncture; **PED** = paroxysmal exercise-induced dyskinesia; **SD** = standard deviation.

Discussion

METAglut1 is an easily performed, robust, and noninvasive diagnostic test for the diagnosis of Glut1DS, which allows wide screening of children and adults, including those with atypical forms of this treatable condition.

Classification of Evidence

This study provides Class I evidence that a positive METAglut1 test accurately distinguishes patients with suspected GLUT1 deficiency syndrome from other neurologic syndromes as compared with invasive and genetic testing.

GLUT1 deficiency syndrome (Glut1DS) is a rare and disabling neurologic disease that can be treated.[1] It is, therefore, of utmost importance to raise awareness about its diagnosis among the medical community. Glut1DS is caused by impaired glucose transport across the blood-brain barrier and into glial cells because of heterozygous, mostly de novo, variants in the *SLC2A1* gene encoding the glucose transporter GLUT1. GLUT1 is a membrane-bound glycoprotein that is particularly abundant in human erythrocytes and brain endothelial and glial cells. Its dysfunction limits brain glucose availability and leads to brain energy deficiency. Besides the classical severe infantile-onset epileptic encephalopathy, Glut1DS also manifests with a wide range of neurologic symptoms in children and adults, including epilepsy, permanent motor disorders, paroxysmal movement disorders, and cognitive impairment, either combined or isolated.[1-3] The early detection of Glut1DS is critical[4] because the disease is treatable with ketogenic diets[5,6] or novel experimental therapies.[7]

A recent work estimates the disease incidence in the general population to be higher than 1 in 24,000.[8] This number only takes into account patients presenting with epilepsy. Some patients present only with movement disorders and/or learning difficulties.[9] Given the number of patients currently identified in registries (e.g., approximately 500–1,000 patients in the United States, 150–200 patients in France, and 60–80 patients in Spain), it is highly likely that a large number of patients with Glut1DS have currently gone undiagnosed. These numbers highlight the importance of tackling underdiagnoses and medical wandering and urge the medical community to improve both awareness about the disease and diagnostic strategy. However, this is challenging for physicians because, on the one hand, the clinical spectrum is very protean, encouraging more frequent testing for Glut1DS, but on the other hand, the current diagnostic strategy relies, as a first step, on an invasive and strict procedure—that is, lumbar puncture (LP) performed in the fasting state with glycemia measured right before LP—followed by genetic analyses (targeted *SCL2A1* analysis or gene panels or whole-exome sequencing). This diagnostic sequence limits the number of patients able to receive the standard of care.

Easy access to a blood biomarker for the early and fast diagnosis of Glut1DS could be determinant for patient outcome and of a major economic effect because earlier treatment is associated with greater patient's prognosis. METAglut1 is a simple test that relies on the quantification of GLUT1 on the erythrocyte surface.[10] It thus provides direct labeling of fresh red blood cells by flow cytometry, similar to routine flow cytometry-based assays in hematology and immunology performed by routine laboratory testing. Only a simple blood draw in an EDTA tube is needed, and it does not require the patient to fast beforehand. A pilot cohort of 30 patients estimated that METAglut1 was 77% sensitive and very specific for Glut1DS.[11] These encouraging results prompted us to evaluate the diagnostic performance of METAglut1. To this end, we conducted a multicenter validation study in France involving 33 centers.

Methods

Standard Protocol Approvals, Registrations, and Patient Consents

This study was registered and approved by ANSM (French Health Authority) and by the ethics committee CPP Ouest V de Rennes (France) under French national identifier ID-RCB 2017-A01473-50. The study was registered on ClinicalTrials.gov under identifier NCT03722212. Written informed consent was obtained from all participants (or guardians of participants) before being enrolled in the study (consent for research).

Study Design

To assess whether METAglut1 has similar diagnostic performances compared with glycorrhachia, we enrolled both a prospective cohort and a retrospective cohort through 33 French participating centers, involving more than 100 neurologists and neuropediatricians. The recruitment started in September 2018 and ended in March 2021.

The prospective cohort consisted of patients presenting with a clinical suspicion of Glut1DS and blind-tested with METAglut1 along with the reference strategy, which consists of a LP for glycorrhachia measurement completed by *SLC2A1* molecular analyses. In the case of an uncertain diagnosis because of potentially discordant results, we further assessed the patient status with an ex vivo functional glucose uptake assay performed on the patient red blood cells. Glycorrhachia, lactatorrhachia, glycemia, and *SLC2A1* molecular analyses were determined at each center under the current standard of care.

The retrospective cohort consisted of patients already diagnosed with Glut1DS based on the reference strategy (i.e., compatible clinical phenotype associated with pathogenic *SLC2A1* variants or hypoglycorrhachia and *SCL2A1* variants of uncertain pathogenicity).

Both cohorts were tested with METAglut1, blind to the patients' condition—clinical, biochemical, and molecular data were not available to the central laboratory performing the test.

Participants

We enrolled both children and adults. Children were older than 3 months because early infantile red blood cells are 20% larger than adult cells with higher GLUT1 levels,[12] which is a confounding factor for the interpretation of GLUT1 expression.

For the prospective cohort, we used the following inclusion criteria: (1) patients with classical phenotypes of Glut1DS: (i) encephalopathy with drug-resistant epilepsy and microcephaly, (ii) early-onset absence epilepsy characterized by EEG, or (iii) generalized epilepsy with a personal or family history of paroxysmal exercise-induced dyskinesia; or (2) patients with atypical forms of Glut1DS defined as unexplained forms (i.e., the absence of argument for an infectious, inflammatory, or tumoral cause) of (1) childhood epilepsy occurring after the age of 4 years characterized by EEG or severe juvenile epilepsy occurring after 10 years characterized by EEG; (2) developmental delay or intellectual disability with a history of epilepsy not yet characterized or a history of drug-resistant epilepsy; (3) paroxysmal movement disorders (pyramidal, ataxic, dyskinetic, ocular), including abnormal movements triggered or aggravated by fasting, exercise, stress, or emotion; (4) permanent movement disorders (pyramidal, ataxic, or dyskinetic) with a history of epilepsy or learning disorders; and/or (5) the patient referred to pediatrics for repeated malaise of unknown origin.

For the retrospective cohort, patients already diagnosed with Glut1DS, based on pathogenic *SLC2A1* variants or hypoglycorrhachia and likely pathogenic *SCL2A1* variants, were eligible. Patients suspected to have Glut1DS with a compatible phenotype, but inconsistent results for the aforementioned parameters (e.g., *SCL2A1* variants of uncertain pathogenicity), and so-called possible Glut1DS were also eligible. For the latter patients, the glucose uptake assay was prescribed to confirm or rule out the diagnosis.

For all patients, exclusion criteria were (1) patients with brain imaging suggestive of a cause other than Glut1DS and (2) situations that could be confounding factors for the interpretation of GLUT1 expression on erythrocytes: (1) patients younger than 3 months; (2) patients with sickle cell anemia, as erythrocytes have higher levels of GLUT1[13]; and (3) patients having undergone a heterologous bone marrow transplant or who had a blood transfusion in the past 120 days because normal erythrocytes can bias the mean GLUT1 expression on the erythrocyte population measured by flow cytometry analysis.

CSF Analyses

CSF glucose and lactate were measured at each center under routine care settings, after at least 4 hours of fasting, along with glycemia, which was measured immediately before the LP.

SLC2A1 Molecular Analyses

SLC2A1 molecular analyses were performed either through direct Sanger sequencing and/or multiplex ligation-dependent probe amplification or through a gene panel tailored for epilepsy or movement disorders or through exome sequencing. Variants were interpreted by geneticists and classified as benign or likely benign (class 1 and 2), variants of unknown significance (VUS) (class 3), probably damaging (class 4), or pathogenic variants (class 5), based on in silico predictive algorithms (CADD, AlignGVGD, SIFT, Polyphen2, MutationTaster and Varsome) with Alamut Visual (Interactive Biosoftware, Rouen, France), frequency in international databases (gnomAD, dbSNP), and segregation data, according to ACMG guidelines.[14]

Erythrocyte Analyses

METAglut1 (METAFORA biosystems, Paris, France) is a CE-marked in vitro diagnostic medical device. The innovation stems from seminal work of academic laboratories and has been turned into an assay which has received the CE mark to facilitate implementation in routine testing laboratories. This test comprises a specially designed assay based on flow cytometry and software for automated computation. A soluble ligand that harbors the receptor-binding domain derived from the HTLV2 envelope glycoprotein (H2RBD) is currently the only reagent that recognizes specifically GLUT1 on red blood cells.[15] It thus provides direct labeling of fresh red blood cells by flow cytometry, similar to routine flow cytometry-based assays in hematology and immunology performed by routine testing laboratories. The sample preparation protocol is minimal, requiring only blood dilution before labeling, followed by washing. Multiple samples can be processed in parallel in a 2-hour run experiment, from sample preparation to results ready to be released. The software includes a series of quality controls of the samples and the run,

Table 1 Clinical Characteristics of All Included Study Participants

Demographic data	Prospective cohort n = 549	Retrospective cohort n = 87
Sex, Female, n (%)	251 (46%)	43 (49%)
Age at inclusion in y, mean (SD)	11,6 (13,1)	13,2 (13,4)
Age at inclusion in classes, n (%)		
[3–24] mo	76 (14%)	3 (4%)
[2–18] y	382 (69%)	56 (64%)
≥ 18 y	91 (17%)	28 (32%)
Inclusion criteria (prospective cohort)		
Classical form, n (%)	156 (28%)	—
Encephalopathy with drug-resistant epilepsy and microcephaly	18 (12%)	—
Early-onset absence eEpilepsy (EOAE) characterized by EEG	136 (87%)	—
Generalized epilepsy with a personal or family history of PED	2 (1%)	—
Atypical form, n (%)	393 (72%)	—
Epilepsy (CAE, JAE, intellectual disability with a history of epilepsy drug-resistant or not)	129 (33%)	—
Abnormal movement (permanent or paroxysmal)	129 (33%)	—
Epilepsy associated with abnormal movement	11 (3%)	—
Other associated atypical forms	124 (31%)	—
Inclusion criteria (retrospective cohort)		
Patients with confirmed Glut1DS diagnosis at inclusion	—	74 (85%)
Patients with pending diagnosis at inclusion (inconsistent biological or genetic data)	—	13 (15%)
Neurologic symptoms at inclusion (prospective) or at diagnosis (retrospective)		
Epilepsy, n (%)	352 (64%)	29 (33%)
Atypical absences	124 (23)	7 (8%)
Partial epilepsy (focal)	26 (5%)	0
Myoclonic epilepsy	23 (4%)	3 (3%)
Tonic/generalized clonic epilepsy	46 (8%)	3 (3%)
Febrile epilepsy	8 (1%)	1 (1%)
Atonic epilepsy	4 (1%)	2 (2%)
Several types of associated epilepsy	120 (22%)	12 (14%)
Not reported	1 (<1%)	1 (1%)
Paroxysmal movements, n (%)	83 (15%)	17 (20%)
Dyskinesia	42 (7%)	8 (9%)
Episodic ataxia	21 (4%)	7 (8%)
Abnormal eye movements	16 (3%)	2 (2%)
Not reported	4 (1%)	0
Epilepsy with paroxysmal movements, n (%)	47 (9%)	28 (32%)
Not reported	67 (12%)	13 (15%)

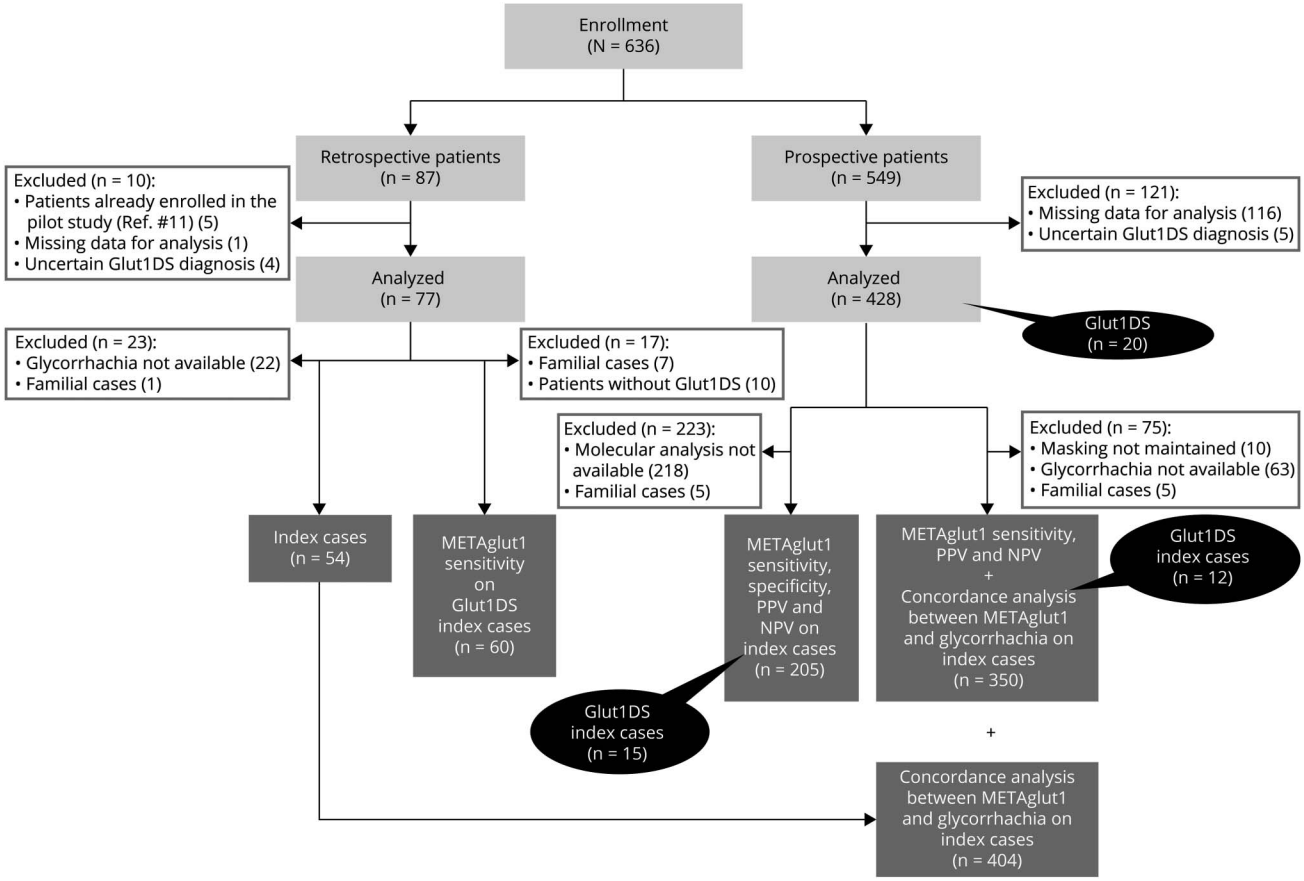
Abbreviations: CAE = childhood absence epilepsy; EOAE = early-onset absence epilepsy; JAE = juvenile absence epilepsy; N = number; PED = paroxysmal exercise-induced dyskinesia; SD = standard deviation.

minimizing time to verify and interpret the data. Altogether the workflow is efficient and ensures a quick turnaround time, which was approximately 48 hours during the study.

METAglut1 was performed in a centralized testing laboratory (Laboratoire CERBA, Saint-Ouen l'Aumône, France), and all functional glucose uptake assays were performed at the *Institut de Génétique Moléculaire de Montpellier* (IGMM, CNRS, France). Both laboratories were blinded to patient's clinical diagnosis or any other biological data when performing the analyses. To avoid bias, the METAglut1 result was also blinded to the investigator until the reference strategy was filed in the eCRF, that is, when the glycorrhachia result was available and molecular analyses were prescribed. This procedure ensured proper and timely clinical management of patients with Glut1DS. Blood samples were collected in EDTA tubes and then sent and stored at 4°C until analyses. The METAglut1 test was performed within 7 days after sampling by the same 4 trained technicians throughout the study. Results were expressed as differences of GLUT1 detection on the cell surface compared with its mean expression across at least 6 samples. Previous results have demonstrated that coefficients of variation were below 5% both in repeatability and reproducibility experiments, allowing for a deployment in routine testing laboratories.

When needed, a sensitive functional glucose uptake assay with red blood cells was performed as described.[16] Once implemented at IGMM, the assay was qualified before the start of the study and demonstrated a mean coefficient of variation of 6% (<8%). Briefly, blood samples were collected in ACD tubes and shipped and stored at 4°C until analyses. The assay was performed at precisely 7 days after sampling for every patient to minimize variability because of potential red blood cell lesions that would occur during storage. Glucose uptake was expressed as % of the mean of uptakes measured with 2–3

Figure 1 Patient Groups Used for the Calculation of Diagnostic Performance



We enrolled 549 patients in the prospective cohort and 87 patients in the retrospective cohort. Within the prospective cohort, 121 participants had to be excluded from the analyses, mainly because METAglut1 was the only available test for these patients (n = 116), with neither available glycorrhachia (lumbar puncture refused by the patients or their caregivers) nor molecular analyses, or because the final diagnosis remained uncertain (n = 5). After excluding mainly patients for whom molecular analyses were not available (n = 218) and a few familial cases (n = 5), 205 index patients were used to compute the sensitivity, specificity, and positive and negative predictive values of METAglut1. These performance criteria were also computed on 350 prospective patients with available glycorrhachia, after excluding 5 familial cases and 10 patients for whom blinding was not maintained—for example, patients for whom glycorrhachia was performed as a confirmatory test after METAglut1 or molecular analyses. Concordance analyses between METAglut1 and glycorrhachia were performed on this same subgroup of patients. Within the retrospective cohort, 10 patients were excluded because of previous enrollment in the initial cohort (n = 5), inconclusive or missing data (n = 1), or uncertain Glut1DS diagnosis (n = 4). From the 77 remaining patients, 60 index patients with Glut1DS were used for sensitivity analysis, after excluding 7 familial cases and revised Glut1DS diagnosis (n = 10). The 54 index patients for whom glycorrhachia was available from the retrospective cohort were used for concordance analyses between METAglut1 and glycorrhachia, after merging them with the prospective cohort of 350 index patients. PPV = positive predictive value; NPV = negative predictive value.

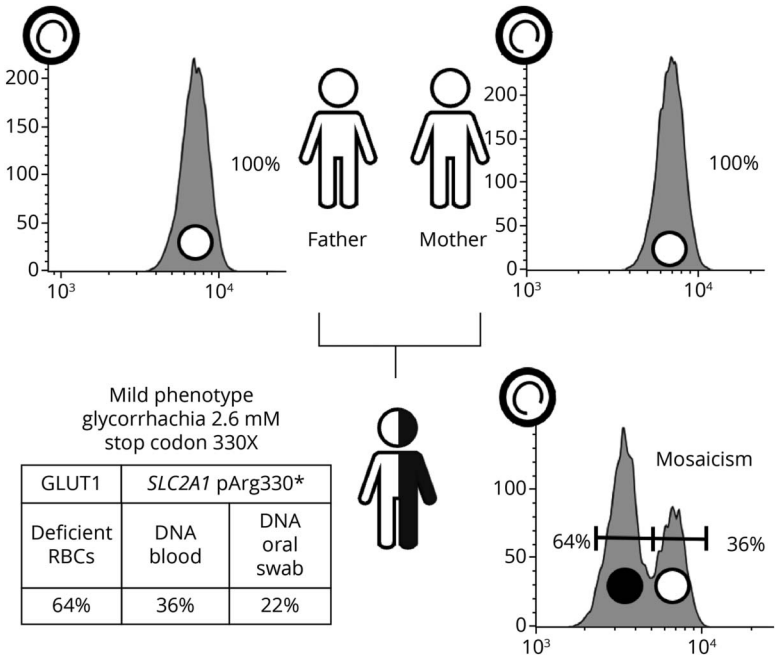
healthy blood donors taken as controls and stored for the same amount of time and in the same conditions. Healthy blood donor samples were provided by the French Blood Center (EFS, Saint-Denis, France) under agreement # 16/EFS/007.

Glut1DS Diagnosis

Criteria for Glut1DS followed the most recent recommendations published by an international Glut1DS working group[17] and were established by an international scientific committee (FM, RP, AGC, SVB, DCD). Glut1DS was confirmed in patients with a compatible clinical phenotype associated with glycorrhachia below 2.2 mM (40 mg/dL) and a pathogenic or likely pathogenic *SLC2A1* variant. Nonetheless, because the normal range of glycorrhachia slightly increases with age,[18] this feature may be more complex to interpret. Therefore, particular attention was given to patients with glycorrhachia comprised between 2.2 (40 mg/dL) and 3 mM (54 mg/dL) and any argument in favor of Glut1DS, such as typical symptoms, or a VUS in the *SLC2A1* gene. In the case of an uncertain diagnosis, an abnormal erythrocyte glucose uptake assay confirmed the diagnosis of Glut1DS. The glucose uptake assay positivity threshold was set at 74% of the controls, as previously reported.[16]

Because METAglut1 is a blood test that can be used as a screening test to improve timely diagnosis, its positive and negative predictive values are critical. Thus, we decided to compare the diagnostic performances of glycorrhachia and METAglut1 using thresholds with high specificity. For CSF, hypoglycorrhachia threshold positivity was set at 2.2 mM (40 mg/dL), because specificity diminishes drastically above

Figure 2 Identification of a Case of Genetic Mosaicism With METAglut1



GLUT1	*SLC2A1* pArg330*	
Deficient RBCs	DNA blood	DNA oral swab
64%	36%	22%

A 12-year-old adolescent patient presented with a moderate phenotype (myoclonic epilepsy, attention, and executive function deficit) and a glycorrhachia of 2.6 mM (47 mg/dL). His red blood cells analyzed with METAglut1 showed an unusual feature with 2 distinct red blood cell populations. One population representing 36% of the patient red blood cells had a normal expression of GLUT1 while the other representing 64% of red blood cells had a distinct lower level of GLUT1 (53% of the controls). Parents of the child were healthy, with normal GLUT1 levels on red blood cells. On deeper sequencing analysis, it was confirmed that 36% of *SLC2A1* copies in the patient's blood DNA and 22% in his oral swab DNA harbored a de novo premature stop codon in the *SCL2A1* gene (pArg330*).

this value,[19,20] along with a CSF/blood glucose ratio below 0.45 and lactatorrhachia below 1 mM (9 mg/dL). For METAglut1, the initial pilot study set a threshold of positivity at a 20% decrease of GLUT1 expression on erythrocytes as diagnostic for Glut1DS.[11] In other words, if a sample showed more than a 20% decrease in GLUT1 expression, it was specific of Glut1DS. This interpretation threshold was chosen a priori for METAglut1 diagnostic performances. *Post hoc* analyses showed that the assay was even more specific at the −24% cutoff. Performance assessment for METAglut1 was thus obtained at the latter refined interpretation threshold.

Data Collection and Security

Data related to patient symptoms were collected within the eCRF on the patient's enrollment, allowing investigators to provide some more details following the specification of the summarized clinical presentation (inclusion criteria). Patients were last recruited in March 2021, and data collection ended in July 2021. A thorough data management plan was implemented with on-site monitoring, automated controls of eCRF, and recoding after queries and data reviewing.

Statistical Analyses

Statistical analyses were performed on all patients selected in the study and whose data were declared usable during a data review and for whom the masking procedure was fulfilled. Statistical analyses were univariate descriptive or based on cross-tabulations. Data management and statistical analyses were performed using SAS V9·4 software (NC) by CEMKA (Bourg-la-Reine, France). The 95% confidence intervals were calculated for the main diagnostic performance criteria and when their estimation was considered necessary. The Cohen kappa coefficient was used for concordance analyses, with null hypothesis kappa = 0 with a one-sided test.[21] Based on an estimated prevalence of 2% among eligible patients for this study, a number of 115 patients would lead to a power of 0.9 with a significance level of 0.05.

Data Availability

The data that support the findings of this study are available from the corresponding authors on reasonable request. Permissions are required to gain access to the data resources, subject to a successful registration and application process.

Results

Patient Groups

We enrolled 549 patients in the prospective cohort: 28% presented with classical phenotypes and 72% had atypical clinical presentations. Sex ratio (determined from preexisting medical records) was close to 1/1, with 46% of females; 14% were infants between 3 months and 2 years, 69% were patients between 2 and 18 years, and 17% were adults. These demographic data were in line with the literature, with no bias toward sex, and a diagnosis that was made mostly in children with a mean age of 7 years.[20] In the retrospective cohort, we enrolled 87 previously diagnosed patients with Glut1DS. Ethnicity data were not available. Table 1 summarizes the clinical characteristics of all included participants. After data collection, review, and analysis by the scientific committee, the 636 enrolled patients were classified as patients either with Glut1DS or not.

Table 2 Clinical and Biological Description of Patients With Glut1DS Not Detected by METAglut1

Patient ID	Cohort	Age at inclusion (y)	Sex	CSF glucose mM*	Glut1 expression %	Glucose uptake %	*SLC2A1* variant# Class	Age at onset and 1st symptom	Epilepsy	Permanent motor disorder	Paroxysmal movement disorder	Cognitive impairment
01	R	10.8	F	**1.8 (32)**	91	*164*	c.94G>C; p.(Val32Leu)[27] Class 4	0.5 y Epilepsy	Generalized tonic-clonic epilepsy	Ataxia	Yes	Attention and executive function deficit
02 Family A index case	P	18.2	F	NA	99	**67**	c.101A>G; p.(Asn34Ser)[28] Class 5	3 y Epilepsy	Generalized tonic-clonic epilepsy	No	No	Moderate ID
03 Family A	P	2.7	M	NA	98	**66**	c.101A>G; p.(Asn34Ser)[28] Class 5	1 y Epilepsy	EAOE and generalized tonic-clonic epilepsy	No	No	Mild ID
04 Family A	P	6.9	M	NA	101	**70**	c.101A>G; p.(Asn34Ser)[28] Class 5	5 y Epilepsy	Atonic seizures	No	No	Mild ID
05	R	7.6	M	**1.6 (29)**	96	NA	c.102T>G; p.(Asn34Lys) Class 5	0.3 y Epilepsy	EAOE and focal epilepsy	No	No	Coordination and attention deficit
06	P	10.4	M	**1.9 (34)**	92	105	c.1300T>G; p.(Phe434Val) Class 4	2 y Epilepsy	EAOE	No	No	Mild ID
07	R	16.7	M	**1.9 (34)**	113	112	c.193T>C; p.(Trp65Arg) Class 4	0.5 y Ocular movement	No	Dystonia	Yes	Mild ID
08	P	56.1	F	**2.1 (38)**	107	100	c.377G>A; p.(Arg126His)[29] Class 5	4 y Psychomotor delay	No	Dystonia and spasticity	No	Mild ID
09	R	21.2	M	**1.8 (32)**	91	**52**	c.376C>T; p.(Arg126Cys)[30] Class 5	2 y Epilepsy	EAOE	Spasticity	Yes	Coordination, attention, and executive function deficit
10	R	17.6	F	**2.1 (38)**	89	77	c.376C>T; p.(Arg126Cys)[30] Class 5	0.5 y Ocular movement	Focal epilepsy	Spasticity (mild)	Yes	Coordination, attention, and executive function deficit
11	R	39.4	M	NA	99	**65**	c.499G>A; p.(Gly167Ser) Class 4	5 y Malaises	No	No	No	Attention and executive function deficit
12	R	12.3	M	NA	112	79	c.493G>A; p.(Val165Ile)[31] Class 5	2.5 y Paroxysmal movement disorder	Atypic absences	No	Yes	Attention and executive function deficit

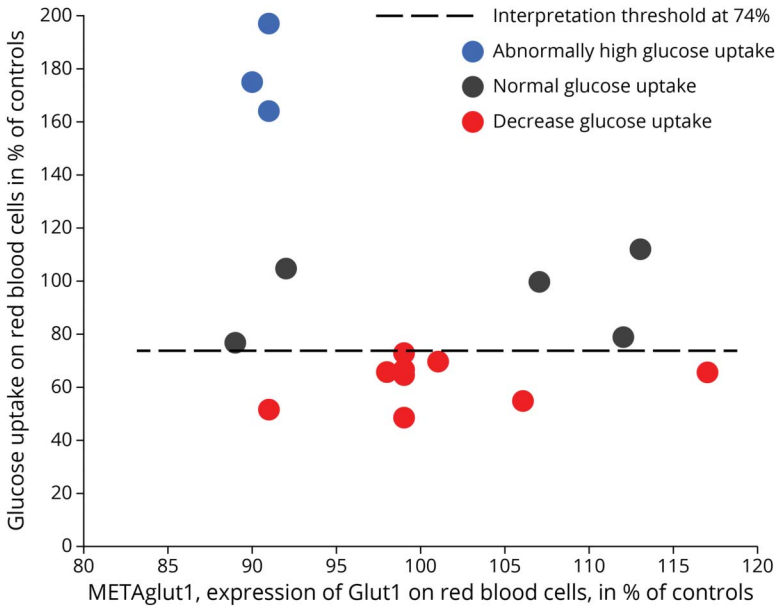
Continued

Table 2 Clinical and Biological Description of Patients With Glut1DS Not Detected by METAglut1 *(continued)*

Patient ID	Cohort	Age at inclusion (y)	Sex	CSF glucose mM*	Glut1 expression %	Glucose uptake %	*SLC2A1* variant# Class	Age at onset and 1st symptom	Epilepsy	Permanent motor disorder	Paroxysmal movement disorder	Cognitive impairment
13 Family B	P	39.9	F	NA	99	**49**	c.539T>A; p.(Met180Lys)[32] Class 4	5 y Paroxysmal movement disorder	No	Ataxia	Yes	Moderate ID
14 Family B index case	P	32.8	F	2.4 (43)	99	**73**	c.539T>A; p.(Met180Lys)[32] Class 4	13 y Paroxysmal movement disorder	No	Ataxia	Yes	Mild ID
15 Family B	P	67.8	M	NA	106	**55**	c.539T>A; p.(Met180Lys)[32] Class 4	2 y Psychomotor delay	No	Ataxia, dystonia	Yes	Mild ID
16	P	4.8	M	**1.9 (34)**	90	*175*	c.884C>T; p.(Thr295Met)[28] Class 5	2 y Psychomotor delay	No	No	No	Speech, coordination, attention, and executive function deficit
17 Family C index case	R	17.3	M	NA	110	NA	c.940G>A; p.(Gly314Ser)[33] Class 5	6 y Paroxysmal movement disorder	No	Dystonia	Yes	No
18 Family C	R	38.6	M	NA	121	NA	c.940G>A; p.(Gly314Ser)[33] Class 5	4 y Paroxysmal movement disorder	No	Dystonia	Yes	Coordination and attention deficit
19	P	11.9	F	2.3 (41)	91	*197*	c.955G>C; p.(Ala319Pro) Class 4	3 y Malaise	No	Ataxia (mild)	Yes	Coordination and attention deficit
20	R	3.7	F	**1.3 (23)**	117	**66**	c.929C>T; p.(Thr310Ile)[34] Class 5	3 y Epilepsy	Myoclonic epilepsy	No	No	Coordination and attention deficit
21	R	3.4	M	**1.9 (34)**	87	NA	c.376C>T; p.(Arg126Cys)[30] Class 5	0.5 y Malaise	Generalized tonic-clonic epilepsy	No	Yes	Coordination and attention deficit

Abbreviations: EAOE = early absence onset epilepsy; F = female; ID = intellectual disability; M = male; NA = non available; in bold, abnormal values for CSF glucose and Glucose uptake; in italics, abnormally high glucose uptake; P = prospective; R = retrospective; y = year.
* In (), values of CSF glucose in mg/dL.
# Previously reported variants.[27-34].

Figure 3 Glucose Uptake Assay



Glucose uptake assay by red blood cells was performed on METAglut1 false-negative patients. The glucose uptake assay interpretation threshold was set at 74% of controls. In 12 index patients with Glut1DS, 4 turned out to have an abnormally low glucose uptake, whereas 5 displayed a normal glucose uptake and 3 an abnormally high glucose uptake.

During the course of the study, a total of 15 index cases (8 with classical phenotypes and 7 with atypical phenotypes) and, overall, 20 patients with Glut1DS (i.e., 3 families of 2–3 patients) were newly diagnosed among the 428 prospective participants for whom glycorrhachia and/or molecular analyses could be obtained (Figure 1). Among these 428 patients, 205 index patients for whom molecular analyses were available were used to compute the sensitivity and specificity of METAglut1 as well as its positive and negative predictive values (Figure 1). Concordance analyses between METAglut1 and glycorrhachia were performed on a larger group of 350 prospective patients for whom both glycorrhachia and METAglut1 were available (Figure 1). Only index cases were used to compute diagnostic performances of the diagnostic test parameters because Glut1DS mostly occurs de novo and familial cases would have introduced bias because of the enrichment of certain *SLC2A1* variants.

Within the retrospective cohort of 87 patients, demographic data were comparable with those of the prospective cohort—a sex ratio of 1/1, 4% of infants between 3 months and 2 years, 64% of patients between 2 and 18 years, and 32% of adults. From the 77 patients with Glut1DS who were retained for analyses, 60 index patients were used for sensitivity analysis, and 54 index patients were used for concordance analyses between METAglut1 and glycorrhachia (Figure 1).

Diagnostic Performance of METAglut1

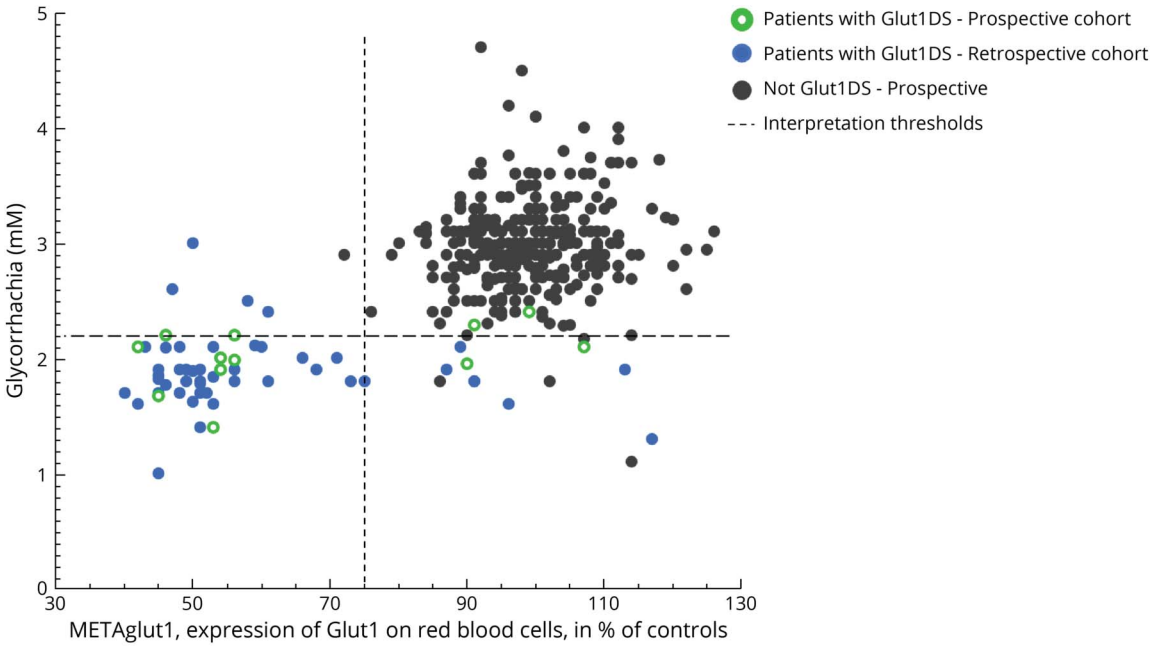
When using a threshold of 76% GLUT1 detection on erythrocytes, METAglut1 sensitivity was found to be 85% (95% CI 76–94) in the retrospective cohort of 60 index patients with Glut1DS. Sensitivity in the prospective group of 205 patients, including 15 index patients with Glut1DS, was 60% (35–85). Specificity was confirmed to reach 99% (98–100), and positive and negative predictive values were 90% (71–100) and 97% (95–99), respectively. A drop in GLUT1 detection greater than 28%, which was the case for most patients, was associated with a positive predictive value of 100%. Overall, METAglut1 was 80% sensitive and >99% specific for the diagnosis of Glut1DS. Notably, METAglut1 allowed us to detect a *SCL2A1* mosaicism in a 12-year-old adolescent patient presenting with a moderate phenotype (Figure 2).

Fifteen of 75 (20%) index patients with Glut1DS were negative for METAglut1. All these patients presented with mild-to-moderate phenotypes (Table 2). The glucose uptake assay could be performed for 12 of these 15 false-negative patients, and 8 had a negative glucose uptake assay (Figure 3). Of note, 3 of these patients had glucose uptake kinetics that were remarkably higher than controls (>160%) while remaining within the normal range for METAglut1 (Table 2). All pathogenic variants associated with METAglut1 false-negative results were missense mutations (Table 2) while nonsense mutations, deletions, and premature stop codons were only found in the METAglut1-positive patients (data not shown).

Comparison of METAglut1 Diagnostic Performance With Glycorrhachia

In our study, the 2.2-mM (40 mg/dL) glycorrhachia threshold matched the low-end value of glycorrhachia in the non-Glut1DS population (Figure 4). When performing calculation on patients with both tests available, the diagnostic

Figure 4 Concordance Analysis Between Glycorrhachia and METAglut1



All patients with both glycorrhachia and METAglut1 available at the time of completion of the study were used to draw the comparative distribution of the 2 biomarkers. The recommended interpretation thresholds are represented with dashed lines at 2.2 mM (40 mg/dL) for glycorrhachia and 76% of normal expression for METAglut1. The Cohen kappa coefficient was computed on the prospective cohort (n = 350 patients)and the whole cohort—350 prospective patients and 48 retrospective patients—for whom both glycorrhachia and METAglut1 were available.

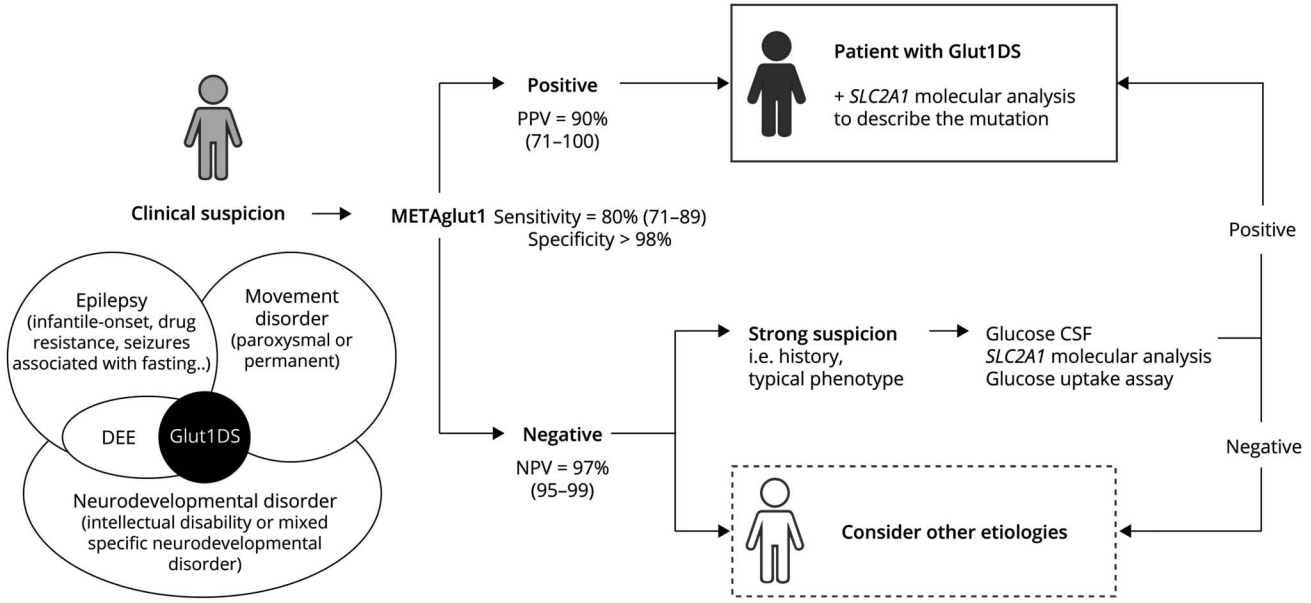
performances of METAglut1 were highly comparable with those of glycorrhachia with a sensitivity of 92% (84–99) for glycorrhachia vs 85% (75–95) for METAglut1 in the retrospective cohort, an equal sensitivity of 67% (40–93) in the prospective cohort, an equal negative predictive value of 99% (98–100), and a positive predictive value of 73% (46–99) for glycorrhachia vs 89% (68–100) for METAglut1. The diagnostic performance of the CSF/blood glucose ratio was similar to that of glycorrhachia. Furthermore, we observed a mean lactatorrhachia of 1 mM (9 mg/dL) (range: 0.7–1.4 mM–6 to 12 mg/dL) for 90% of patients with Glut1DS, compared with a mean of 1.4 mM (12 mg/dL) (range: 0.8–1.9 mM–7 to 17 mg/dL) for 90% of patients without Glut1DS, supporting that CSF lactate is low-normal to low in Glut1DS.[18]

The small number of prospective patients, which is inherent to a rare disorder, did not allow us to perform statistical comparisons between the sensitivity of the 2 tests. Nevertheless, we applied a concordance analysis to further match the 2 diagnostic tests. METAglut1 and glycorrhachia were compared on the subset of 350 prospective patients for whom both tests were available. The 2 tests were in agreement, with a Cohen kappa coefficient of 0.59 (0.33–0.85; significantly better than 0 with $p = 0.004$) (Figure 4). As Glut1DS is a rare disease, the large number of double negative cases (336) imbalances the matrix (6 double positives and 5 vs 3 single positives), explaining the only moderate-to-strong agreement of 0.59 along with a wide 95% CI in the prospective cohort. Because both the prospective and retrospective cohorts have been analyzed by the same central laboratory unbeknownst of their status, we also performed concordance analyses in the cumulated cohort. The Cohen kappa coefficient then reached 0.78 (0.69–0.87; significantly better than 0 with $p < 0.001$), which is considered as a substantial agreement (341 double negatives, 43 double positives, and 13 vs 7 single positives). The mean value falls close to the 0.8 threshold of Cohen kappa coefficient to state an almost perfect agreement. The low range of the 95% CI matched 0.7 that is classically considered as a good concordance, and its upper range encompassed values that are considered as an almost perfect agreement.

Discussion

We demonstrated that the METAglut1 blood test can expedite the diagnosis of Glut1DS with 80% sensitivity and >99% specificity. Those values are comparable with the 77% sensitivity found in the pilot cohort.[11] Moreover, this multicenter prospective cohort allowed to estimate the positive and negative predictive values of METAglut1, which reached 90% (71–100) and 97% (95–100), respectively. We identified 20 patients with Glut1DS in the prospective cohort, which is in

Figure 5 Proposed New Diagnostic Algorithm for Glut1DS Diagnosis in the Standard of Care



Positive and negative predictive values of METAglut1 are those obtained from the prospective cohort, with all patients with confirmed Glut1DS, whether they had glycorrhachia available. Glut1DS = Glut1 deficiency syndrome; DEE = developmental and epileptic encephalopathy; Se = sensitivity; Spe = Specificity; PPV = positive predictive value; NPV = negative predictive value; CSF = cerebrospinal fluid.

line with previous reports.[22] Indeed, the occurrence of Glut1DS within the so-called classical clinical forms has been estimated between 5% and 10%, whereas 1% to 2% of patients with Glut1DS are expected among the so-called atypical clinical forms.[23] Our study enrolled 156 patients with a classical phenotype, which would translate into an expected 7 to 16 patients among this population, and 393 patients with an atypical phenotype, corresponding to an expected 4 to 8 patients with Glut1DS. Thus, a total of 11–24 patients were expected in the prospective cohort, which compares very favorably with the 20 patients with Glut1DS whom we diagnosed in our study. Accordingly, the cohort used in this study can be validated as representative for Glut1DS.

Because hypoglycorrhachia is a hallmark of Glut1DS and that LP is used as a first test in the current diagnostic strategy, we wished to compare glycorrhachia levels with METAglut1. In the retrospective and prospective cohorts, the diagnostic performances of both tests were highly similar in sensitivity, specificity, and positive and negative predicted values. They also demonstrated good concordance at the patient level. A slightly higher sensitivity was found for glycorrhachia in the retrospective cohort compared with METAglut1 (92% vs 85%, respectively). Such a bias was expected because most patients in this cohort were previously diagnosed based on hypoglycorrhachia. Moreover, our study allowed us to determine the positive predictive value of glycorrhachia, which appeared (even at the 2.2 mM/40 mg/dL threshold) to be lower than that of METAglut1 (73% vs 89%, respectively).

Our study confirmed that 1 Glut1DS patient of 5 was negative for METAglut1. Among these patients, we found that two-thirds (8/12) were also negative for the glucose uptake assay, a very sensitive functional assay, which is considered the gold standard for assessing Glut1DS. Notably, the best cutoff values are equivalent for both erythrocyte tests, with approximately 74%–76% of the controls, and is likely because of similar biological parameters measured by the 2 assays. These findings highlight important diagnostic challenges in some patients with Glut1DS. A possible explanation for METAglut1 false-negative results is that mutations are likely to bear different consequences on GLUT1 function in erythrocytes than they do on the blood-brain barrier, in part likely because of tissue-specific GLUT1 partners that can distinctively modulate the effect of the mutation on the erythrocyte surface. Because METAglut1 relies on the quantification of GLUT1 presence on the erythrocyte surface by the H2RBD-specific ligand,[24] mutations that do not prevent either its binding or the transporter to be properly expressed on the cell surface, whether this stems from a problem of translation or folding or trafficking, are likely to remain undetected. This observation suggests that METAglut1 false negatives reflect rather an altered glucose uptake kinetics than an effect on the actual presence of the protein on the cell surface. Accordingly, it appeared that all false-negative patients harbored missense mutations and presented with milder phenotypes. Although it is not a definitive conclusion, these mutations may only partially affect glucose transport, triggering moderate phenotypes in patients.

Our study validates prospectively and thoroughly a circulating biomarker against the reference diagnostic strategy that includes glycorrhachia. The very good diagnostic performance of METAglut1 in our multicenter cohort, which is the largest

reported for this disease, reinforces the clinical relevance of this test, which can be easily and rapidly used by prescribers. Unfortunately, this study did not allow us to formally establish the diagnostic time gained with METAglut1 because most investigators opted to run both tests in parallel during inpatient clinics for practical reasons. With a typical turnaround of 48 hours, the test is robust with few retests necessary, thus warranting an easy adoption in routine clinical settings, notably in outpatient clinics. METAglut1 can be proposed as a first-line investigation to test for Glut1DS without the constraints of a spinal tap, fasting, or expertise to interpret metabolic changes related to age.[18] Incidentally, but quite illustratively, this study led to diagnose a few patients with Glut1DS who, many years earlier, had a LP displaying hypoglycorrhachia, but that went unnoticed, therefore leading to major delays in diagnosis and treatment. The specificity of METAglut1 for Glut1DS is also of great added value, unlike hypoglycorrhachia that can be also caused by hypoglycemia, meningitis, subarachnoid hemorrhage, or ventriculoperitoneal shunt systems. The high positive predictive value of METAglut1 supports its use as a screening test for Glut1DS on patients because a positive result is actionable by triggering early treatment and, therefore, likely to greatly improve prognosis.[4] Furthermore, although next-generation sequencing (NGS) methodologies keep expanding worldwide, only a few countries can offer NGS as first-line investigations for the diagnosis of rare diseases, mainly in pediatrics. At best, the turnaround time is of several weeks to several months—as illustrated in our study with a large number of unavailable molecular analyses—which is not desirable for epileptic children with Glut1DS. Moreover, the interpretation of *SCL2A1* variants can be quite challenging, with frequent missense variants and a great proportion of VUS. In our study, METAglut1 turned positive for 3 patients with *SLC2A1* VUS. Likewise, METAglut1 can provide critical information regarding puzzling molecular analyses such as VUS or genetic mosaicism.

Glut1DS is an urgent diagnosis for patients, not to miss critical time where early treatments can be initiated to support brain development and function.[25] To this end, we suggest to perform METAglut1 in any patient, after the age of 3 months, who presents with intellectual disability or mixed specific neurodevelopmental disorder, epilepsy (especially drug-resistant or ketogenic diet-responsive), deceleration of head growth, permanent movement disorders (cerebellar ataxia, dystonia, or spasticity), and/or paroxysmal movement disorders (Figure 5). If performed at an early symptomatic stage, this simple test will identify 80% of patients with Glut1DS right away among those with developmental and epileptic encephalopathy,[26] intellectual disabilities, or movement disorders. The high positive predictive value (90%) of METAglut1 is of paramount importance for the diagnosis of Glut1DS and the initiation of dedicated treatments as soon as possible. Moreover, the good negative predictive value of the test (>95%) can be sufficient to rule out Glut1DS in most cases. However, in the case of a negative result but a strong clinical suspicion, measurement of glycorrhachia and/or *SLC2A1* molecular analyses are warranted to further explore the possibility of Glut1DS (Figure 5). Given the current estimated prevalence of Glut1DS, the availability of a simple blood test is a major milestone for patients with Glut1DS because their diagnosis and treatment will occur much faster.

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Disclosure

F. Mochel serves on the scientific advisory board of Metafora biosystems; METAFORA biosystems is a startup that developed METAglut1; D. Gras reports no disclosures relevant to the manuscript; MP. Luton reports no disclosures relevant to the manuscript; M. Nizou is an employee at Metafora biosystems; D. Giovannini reports no disclosures relevant to the manuscript; C. Delattre reports no disclosures relevant to the manuscript; M. Aubart reports no disclosures relevant to the manuscript; M. Barth reports no disclosures relevant to the manuscript; A. De Saint-Martin reports no disclosures relevant to the manuscript; D. Doummar reports no disclosures relevant to the manuscript; N. Essid reports no disclosures relevant to the manuscript; A. Garros reports no disclosures relevant to the manuscript; C. Hachon-Le Camus reports no disclosures relevant to the manuscript; C. Hoebeke reports no disclosures relevant to the manuscript; S. Nguyen The Tich reports no disclosures relevant to the manuscript; M. Périvier reports no disclosures relevant to the manuscript; S. Rivera reports no disclosures relevant to the manuscript; A. Rolland reports no disclosures relevant to the manuscript; A. Roubertie reports no disclosures relevant to the manuscript; C. Sarret reports no disclosures relevant to the manuscript; C. Sevin reports no disclosures relevant to the manuscript; D. Ville reports no disclosures relevant to the manuscript; M. Sitbon is the inventor of a patent describing the use of the H2RBD ligand for the evaluation of GLUT1 expression; he is the cofounder of Metafora biosystems and head of the scientific advisory board; JM. Costa is biologist at Cerba Healthcare, a testing laboratory implementing the Metaglut1 assay for the routine; R. Pons reports no disclosures relevant to the manuscript; A. Garcia-Cazorla reports no disclosures relevant to the manuscript; S. Vuillaumier-Barrot reports no disclosures relevant to the manuscript; V. Petit is a cofounder and CEO of

Metafora biosystems; O. Boespflug-Tanguy reports no disclosures relevant to the manuscript; and D. C. De Vivo serves on the scientific advisory board of Metafora biosystems. Go to Neurology.org/N for full disclosures.

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Appendix 1 *(continued)*

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Appendix 2 Coinvestigators

Coinvestigators are listed at links.lww.com/WNL/C767.

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