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Research report

In-vivo brain H1-MR-Spectroscopy identification and quantification of 2-hydroxyglutarate in L-2-Hydroxyglutaric aciduria

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ABSTRACT

L-2-Hydroxyglutaric aciduria (L2HGA) is an extremely rare hereditary neurometabolic disease, characterized by increased L-2-hydroxyglutarate (L2HG) levels in the brain and biological fluids. 24-h urine 2HG level remains the biochemical hallmark for the diagnosis of L2HGA, whereas it is unknown the feasibility to measure *in vivo* the intracerebral levels of 2HG by using magnetic resonance spectroscopy (MRS).

Patients and methods: We used at 3T H^1-MRS Single-Voxel (SV) PRESS sequences tailored to detect 2HG, in three adult patients with the diagnosis of L2HGA and in healthy controls. We also used mass spectrometric methods to measure the levels of 2HG in plasma and serum.

Results: 2HG peak was detected and quantified in the white matter (WM) of the three L2HGA patients, while it was absent in controls. All patients showed also high levels of 2HG in plasma and serum.

Conclusions: Brain 2HG detected by MRS may play a role in the diagnosis and follow-up of L2HGA, besides circulating plasma/serum 2HG levels by mass spectrometric assays, although studies on a large cohort of patients are required to confirm these observations.

1. Introduction

L-2-Hydroxyglutaric aciduria (L2HGA, OMIM: 236792) is a very rare autosomic recessive metabolic disorder affecting exclusively the central nervous system. Neurological manifestations include mental retardation and a variety of motor symptoms, including ataxia, pyramidal and extrapyramidal manifestations. The evolution can be slowly progressive; mild form of L2HGA can be undiagnosed till adulthood. Increased occurrence of brain tumors has been reported (Haliloglu et al., 2008; Moroni et al., 2004). Mutations in gene encoding L-2-hydroxyglutarate dehydrogenase (*L2HGDH*), the enzyme that catabolizes L-2-hydroxyglutarate (L2HG) to 2-ketoglutarate, lead to accumulation of L2HG, normally maintained at a very low level in tissues (Kranendijk et al., 2012). The diagnosis of L2HGA can be made at three levels (Steenweg et al., 2010). First, by conventional magnetic resonance imaging (cMRI) that shows abnormalities predominantly of subcortical white matter (WM) in combination with dentate, lenticular and caudate nuclei alterations, associated to cerebral and cerebellar atrophy (Steenweg et al., 2009). Second, by the presence of increased L2HG levels in 24-h urine samples. Third, by identification of *L2HGDH* mutations, scattered along the gene without any exonal preference (Kranendijk et al., 2012).

Abbreviations: ADC, Apparent Diffusion Coefficient; cMRI, conventional magnetic resonance imaging; CRBL, Cramér-Rao lower bounds; CSF, cerebro-spinal fluid; DWI, Diffusion weighted images; EDDS, Expanded Disability Status Scale; FLAIR, Fluid-Attenuated Inversion Recovery; L2HG, L-2-hydroxyglutarate; L2HGA, L-2-Hydroxyglutaric aciduria; L2HGDH, L-2-hydroxyglutarate dehydrogenase gene; IDH, Isocitrate-Dehydrogenase 1/2; MRM, multiple reaction monitoring mode; MRS, Magnetic resonance spectroscopy; NAA, N-acetyl-aspartate; ROI, Region of Interest; ppm, parts per million; PRESS, Point Resolved Sequence; SV, single-voxel; TCA, Trichloroacetic acid; TE, Echo Time; TQD, triple quadrupoles mass spectrometer; UPLC, Ultra High Pressure Liquid Chromatography system; w.i., weighted images; WM, white matter; 2HG/Creatine, 2HG/Cr

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Magnetic resonance spectroscopy (MRS) plays an important supportive role to cMRI in the diagnosis of metabolic disorders (Rossi and Biancheri, 2013). It has the capability to detect and quantify *in vivo* metabolites in the brain, based on the amplitude of their characteristic resonance frequencies peaks. 1H-MRS has become widely available in a routine clinical setting because hydrogen is the most abundant atom in the human body and the related signal-to-noise ratio allows to detect the main metabolites of clinical interest at 1.5T yet and to obtain more detailed high resolution spectra at higher magnetic field strength (McRobbie et al., 2006).

MRS findings were reported in few L2HGA cases (Aydin et al., 2003; Sener, 2003; Read et al., 2005), resulting in prominent multiplet resonating at 2.1–2.5 ppm, possibly attributable to glutarate or glutamate/glutamine, with no identification of 2HG peak. The intracerebral accumulation of 2HG cannot be easily detected by MRS (Aydin et al., 2003; Sener, 2003; Read et al., 2005), because of the structural complexity of the molecule that gives raise to multiplets resonating at three frequencies at 3T (located at 1.9, 2.25 and 4.02 ppm (parts per million) being the 2.25 ppm larger than the other 2HG peaks and the 4.02 ppm the less detectable) whose spectrum overlaps those, greater, of N-acetyl-aspartate (NAA) and glutamate (Bertolino et al., 2014; Choi et al., 2013). Specifically tailored MRS sequences have been recently implemented to identify and quantify *in vivo* 2HG in patients affected by Isocitrate-Dehydrogenase (IDH) 1/2 mutated gliomas, as IDH1/2 mutations lead to 2HG accumulation in the tumor (Choi et al., 2012; Andronesi et al., 2013).

Here we report the detection of 2HG in the brain of three patients with diagnosis of L2HGA by using 3T MRS, and its detection in their urine and plasma/serum by using mass spectrometric methods.

2. Results

Three adult patients with L2HGA were investigated by brain MRI and MRS, biochemical (2HG detection in urine, plasma and serum) and genetical analysis. The diagnosis was suggested by brain cMRI (prominent subcortical WM T2-weighted and FLAIR hyperintensity, with central grey and dentate nuclei involvement) and confirmed by the increased 2HG urine level and *L2HGDH* gene analysis (Table 1).

The patients are 61 (patient A), 39 (patient B), 24 (patient C) years-old, and show different severity of disease. In particular, clinical performance was grossly synthetized by Expanded Disability Status Scale (EDSS) (Kurtzke, 2015), and described as following: patient A: EDSS: 2; mild learning disability with adult-onset behavioral and cognitive decline, and choreic movements; patient B: EDSS: 6.5; psychomotor delay, seizures, and progressive ataxia, extrapyramidal and pyramidal features (wheelchair-bound at the age of 34 years); patient C: EDSS: 3; psychomotor delay, with mild, non-progressive extrapyramidal, pyramidal, and ataxic features. cMRI patterns are showed in Fig. 1A–C.

Molecular analysis of *L2HGDH* gene revealed the presence of novel pathogenic mutations: in patient A, the homozygous missense variation c.844C > T (p. Arg282Trp), not described in literature and not reported in the Exome Variant Server Database; in patient B, the nonsense variation in homozygous form c.339T > A (p. Cys113*), not previously described in association with the pathology and not reported in the Exome Variant Server Database, and the novel heterozygous missense c.320C > A (p. Ser107Tyr); in patient C, the novel homozygous frameshift variation c.530delC (p. Pro177Hisfs*6). Besides, in patient C we found two other homozygosis variants: the missense variant c.533A > T (p. Tyr178Phe), described in ExAC database with an allele frequency of 4/121392 controls and the missense c.53T > C (p. Leu18Arg), described in dbSNP (rs2275591) with an allele frequency of 0.60 (details in Supplementary Materials).

2HG was clearly detected within the spectra in all L2HGA patients by means of the 2HG-tailored sequences (Fig. 2, Table 1) while not distinguishable by means of standard Point Resolved Sequence (PRESS) sequence.

2HG levels quantified at Echo Time 30 (TE30) were higher and Cramér-Rao lower bounds (CRLB) was lower (except for one case, patient A) than those at TE97 (Table 1, Fig. 2).

2HG peaks were not detectable in controls (Fig. 2).

2HG was determined in plasma and serum of each L2HGA patient, as well as excreted into urine, and was invariably high in L2HGA patients; 2HG was not detected in controls.

No clear relation was found between 2HG level and disease severity. Nevertheless, the less compromised patient (pt A) had lowest 2HG levels as detected by MRS at TE30 and, at the same extent, in blood and urine samples, while the patient with the most severe neurological impairment (pt B) showed the largest brain 2HG peak (as detected by MRS at TE30) (Table 1).

Apparent Diffusion Coefficient (ADC) values in the voxel site were higher in L2HGA patients (mean ± sd 1470 ± 199.1, range 1241.6–1605.7) than in controls (mean ± sd 867.3 ± 15.7, range 849.2–877.3).

No L2HGA patient exhibited brain tumors on MRI.

Table 1
Genetic and biological data relative to the three L-2-Hydroxyglutaric aciduria patients. *L2HGDH* gene mutation and 2HG urine level confirmed L2HGA diagnosis for each patient (A, B, C). At the bottom brain 2HG level (and 2HG/Cr) detected by MRS and plasma/serum 2HG levels detected by mass spectrometry are reported. Abbreviations: MRS = magnetic resonance spectroscopy.

Subject identification		**Patient A**	**Patient B**	**Patient C**	Healthy controls
Urine 2HG level (μg/mg creatinine)		217	733	459	< 40
L2HGDH gene mutation ([a])		p.Arg282Trp/p.Arg282Trp	p.Cys113*/p.Ser107Tyr + p.Cys113*	p.Pro177Hisfs*6/p.Pro177Hisfs*6	–
Brain MRS					
TE = 30 ms	2HG level (mM)	2.238	4.316	3.433	Not detectable
	2HG/Cr	0.746	0.971	1.151	
TE = 97 ms	2HG level (mM)	1.923	1.873	1.687	Not detectable
	2HG/Cr	0.499	0.362	0.532	
Blood 2HG level (uM)					
Serum		16.1	48.39	55.45	< 0.5
Plasma		16.2	47.32	55.41	< 0.5

[a] See text and Supplementary.