L-2-Hydroxyglutaric aciduria in two Palestinian siblings with a novel mutation in the *L2HGDH* gene

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Abstract

$_L$-2-Hydroxyglutaric aciduria is a rare autosomal recessive neurometabolic disorder caused by deficiency of $_L$-2-hydroxyglutarate dehydrogenase. This enzyme catalyses the conversion of $_L$-2-hydroxyglutarate to alpha-ketoglutarate and its deficiency causes accumulation of $_L$-2-hydroxyglutarate which is s toxic to the brain leading to the leukoencephalopathy.

We report a novel mutation in the L2HGDH gene in two siblings with $_L$-2-Hydroxyglutaric aciduria and describe clinical phenotype. They presented with developmental delay, cerebellar ataxia, tremor and speech regression. Urine organic acid analysis revealed massive excretion of 2-Hydroxyglutaric acid. Brain magnetic resonance imaging showed the characteristic leukodystrophy involving the subcortical cerebral white matter and dentate nucleus, sparing the basal ganglia. Genetic analysis of L2HGDH gene showed that they were homozygous for the novel mutation chr14:50,750, 731 c.560_561insATTG (p.C187XfsX1) in exon 5 of L2HGDH gene. Both patients showed symptomatic response to riboflavin and levocarnitine therapy.

Conclusion: This report further expands the genetic spectrum of $_L$-2-HGA and suggests a successful response to treatment with levocarnitine and riboflavin

Keywords: *$_L$-2-Hydroxyglutaric aciduria, L2HGDH gene, white matter, leukoencephalopathy, ataxia*

Introduction

L-2-Hydroxyglutaric aciduria (L-2-HGA) is a rare autosomal recessive neurometabolic disorder affecting mainly the central nervous system. It is caused by mutations in *L2HGDH* gene that encodes L-2-hydroxyglutarate dehydrogenase (Augoustides-savvopoulou et al, 2007; Kranendijk et al, 2012). This enzyme catalyses the conversion of L-2-hydroxyglutarate to alpha-ketoglutarate (Van Schaftingen et al, 2009). L-2-hydroxyglutarate is toxic to the brain, causing a leukoencephalopathy and increases the susceptibility to develop tumors (Van Schaftingen et al, 2009; Steenweg et al, 2009).

Typically, the disease follows a progressive neurodegenerative course. Common features include cerebellar ataxia, mental retardation and learning difficulties (Sass et al, 2008). Other frequently reported manifestations include macrocephaly, seizures, dysarthria and pyramidal and extrapyramidal signs (Sass et al, 2008; Larnaout et al, 2008).

The combination of involvement of subcortical cerebral white matter MRI abnormalities that spare the deep white matter (WM) and the corpus callosum, in addition to the involvement of dentate nuclei and atrophy of the cerebellar vermis has been considered pathognomonic for L-2-HGA (Sass et al, 2008; Fourati et al, 2016). Currently, 86 mutations in *L2HGDH* gene have been described in 164 individuals, The majority of which are missense mutations that alter invariably conserved amino acids (Kranendijk et al, 2012; Sass et al, 2008).

We report two siblings diagnosed with L-2-HGA and presenting with the classical phenotype and the characteristic leukoencephalopathy that was limited to subcortical white matter and dentate nucleus. They were homozygous for the novel mutation chr14:50,750,731 c.560_561insATTG (p.C187XfsX1*)* in exon 5 of *L2HGDH* gene which is a duplication mutation of ATTG at codon 187 that results in frame shift and premature termination of protein translation at the amino acid residue 187. Family segregation studies of the *L2HGDH* gene and direct sequencing was performed on the DNA extracted from peripheral blood samples (Augoustides-savvopoulou et al, 2007; Sass et al, 2008) showed that both parents and two brothers were heterozygous for the same mutation and two sisters were homozygous for the wild type allele.

We hypothesize a possible correlation between the genotype, phenotype and the extent of leukoencephalopathy in our patients, but correlation between genotype and phenotype requires future studies.

This study was approved by the ethics committee of Makassed Hospital. Written informed consent was obtained from the parents granting permission for genetic analysis, brain MRI imaging and publication.

Case Presentation

Case 1

A 15-year-old male who was the third child of 4 offsprings of healthy consanguineous parents (first cousins). He was born at term after normal pregnancy and labour. Birth weight was 3.5 kg (+1 SD), length and head circumference at birth were not recorded. The patient's clinical course was uneventful apart from speech delay that was noted after age of 2 years. After age 6 years, he developed progressive mental deterioration, ataxic gait, truncal ataxia, hand tremor and interrupted slow speech. Several anticonvulsant medications were given without improvement. Brain MRI performed at age 8 years and 11years revealed leukodystrophic changes which were not further investigated.

He was hospitalized at our center at age 15 years for evaluation of mental retardation, ataxia, tremor and progressive difficulty of speech. His weight was 46 kg (-1.37 SD), height 160 cm (-1.3 SD) and head circumference 54 cm (-0.65 SD). Neurological examination revealed ataxic gait, dysarthria, dysmetria, dysdiadochokinesia, hand tremor, lower limb spasticity and exaggerated deep tendon reflexes in both lower limbs with ankle clonus. Metabolic workup included: Plasma ammonia 30 µmol/L (normal 11-50 µmol/L), lactate 1.7 mmol/L (normal 0.5-2.2 mmol/L), homocysteine 4 µmol/L (normal < 20 µmol/L). Urine organic acid analysis revealed marked excretion of 2-HGA (qualitative). Plasma amino acids including plasma lysine were normal. Other laboratory findings included normal thyroid function tests, plasma uric acid, kidney and liver function tests. Brain MRI images showed high signals involving subcortical white matter and dentate nucleus (Figure 1). Treatment included levocarnitine orally 75 mg/kg/day and riboflavin orally100 mg three times daily.

Case 2

An 8-year-old female, the younger sister of patient 1, was also born at term after normal pregnancy and labour. Birth weight was 3.5 kg (+1 SD), length and head circumference were not recorded. The patient's clinical course was characterized by speech delay noted at age 2 years. At age 3 years, she developed truncal ataxia and hand tremor. First hospitalization was at age 7 years for evaluation of progressive mental deterioration, hyperactivity and worsening of ataxia and tremor noted after age 6 years. Her weight was 30 (+1.44 SD), height 130 cm (+ 1.4 SD) and head circumference 52 cm (+0.47 SD). Neurological examination revealed ataxic gait, dysarthria, dysmetria and exaggerated deep tendon reflexes in both lower limbs. Metabolic workup included: Plasma ammonia 25 µmol/L (normal 21-50 µmol/L), lactic acid 2.4 mmol/L (normal 1-2.4 mmol/L). Plasma amino acids including lysine were normal. Urinary organic acid assay also revealed marked excretion of 2-HGA (qualitative). T2-weighted brain MRI also showed high signals involving the subcortical white matter and dentate nucleus (Figure 2). Treatment included Levocarnitine orally 100 mg/kg/day and riboflavin orally 100 mg three times daily. The family reported improvement in ataxia and speech in both patients after 6 months of therapy but the parents denied re-hospitalization for clinical and biochemical evaluation.

Methods and Materials of DNA sequencing

DNA extraction: Genomic DNA was extracted from whole peripheral venous blood, using a commercially available kit (MasterPure™ Complete DNA & RNA Purification Kit, Cat No.MCD85200).

Polymerase chain reaction (PCR): Polymerase chain reaction amplification and DNA sequencing were used to screen exon 5 to detect the presence of the mutation in this family.

A total reaction 50µl for PCR containing; 25µl of ready mix GO Taq green master mix (Promega), 0.1 µg/µl of genomic DNA, 4% primers (F/R) and water. DNA amplification was done by using these primers L2HGDH-5F: gaagaaaagcttggcaaaatc and L2HGDH-5R: caaaacccatggatatggag. The PCR cycling conditions included preheating for 5 minutes at 95°C, followed by 35 cycles of 95°C for 30 seconds annealing at 60°C for 30 seconds 72°C for 30 seconds and a final extension 5 minutes at 72°C.

DNA Sequencing: DNA sequencing was performed on an ABI sequencer and results compared to the wild type sequence (accession: NG_008092.1) using the Mutation Surveyor software.

The result of the molecular genetic analysis showed that both patients were homozygous for the mutation chr14:50,750,731 c.560_561insATTG (p.C187XfsX1*)* in exon 5 of *L2HGDH* gene. Family segregation studies showed that both parents and two brothers were heterozygous for the same mutation and two sisters were homozygous for the wild allele.

The c.560_561insATTG mutation is in exon 5 of the L2HGDH gene is an insertion and duplication of the ATTG nucleotides at codon 187 that leads to frame shift and premature termination of protein translation (p.C187XfsX1).

We believe that is variant is a pathogenic mutation because it leads to a frameshift with premature termination of protein synthesis after one codon (Figure 3). In addition, we tested the pathogenicity of this variant by Mutation Tasters software and the prediction was that it is a disease causing mutation. Furthermore, analysis of the Human Genome Database (HGD) indicated that there is a frameshift mutation that was reported in the database at an adjacent amino acid.

Discussion

The two siblings in our report were homozygous for the novel mutation chr14:50,750,73 c.560_561insATTG (p.C187XfsX1*)* in exon 5 of *L2HGDH* gene. This genotype correlated with the phenotype, leukodystrophy on brain MRI and the biochemical finding of increased urine level of 2-HGA in both patients. Both patients had identical phenotype consisting of cerebellar ataxia, pyramidal syndrome and mental retardation. They also had white matter MRI abnormalities that were limited to subcortical white matter and the dentate nuclei.

Molecular genetic heterogeneity in the *L2HGDH* gene was reported in a series of 106 patients belonging to 83 families (Steenweg et al, 2010). The study reported 53 mutations, 35 of them were novel and the majority were missense variants that invariably alter conserved amino acids. The main manifestations were developmental delay, cerebellar ataxia, epilepsy and macrocephaly.

Characteristically, in patients with progression of the disease, WM changes and basal ganglia signal involvement become more diffuse resulting in atrophy of the cerebral WM atrophy (Steenweg et al, 2009; Fourati et al, 2016). A retrospective study of 14 brain MRI studies performed in 10 patients with L-2-HGA showed extensive confluent subcortical signals with symmetrical involvement of dentate nuclei and basal ganglia (Sudhakar et al). In our report, patient 1 did not show spread of WM abnormalities at age 16 years and both patients did not show involvement of basal ganglia. This finding may represent a genotype-phenotype correlation, or represent one end of the spectrum of MRI abnormalities in this disorder. However, because there are so many different genotypes, it is not possible to define a specific phenotype associated with each mutation.

Treatment of a 40-year –old female with L-2-HGA who had limb spasticity, tremor, severe dystonia involving the neck and arms, and mild cognitive delay with levocarnitine and FAD resulted in improvement of dystonia and tremor and normalization of gait and decreased urinary excretion of L-2-Hydoxyglutaric acid after 6 months of treatment. The clinical improvement was maintained for more than 4 years following after the start of therapy (Samuraki et al, 2008). Successful therapy was also reported with riboflavin (100 mg daily) in a 16-year-old boy resulting in partial improvement of cognitive and motor function within days of treatment. Decreased excretion of L-2-hydroxyglutaric acid was noted after 3 months of treatment (Yilmaz et al, 2009). Interruption of riboflavin treatment was followed by recurrence of symptoms and increased urinary excretion L-2-hydroxyglutaric acid.

In contrast to these reports, a 9-year-old female with L-2-HGA developed progressive tremor, ataxic gait, dysarthria and mental retardation despite treatment with riboflavin (200 mg/day) (Jequier et al, 2008). She had persistent of L-2-hydroxyglutaric acid in body fluids after two months of treatment. Her sister died suddenly at the age of 11 months during intercurrent illness. Both were homozygous for a splice site mutation in *L2HGDH* gene.

Our patients showed partial improvement of ataxia and speech after 6 months of treatment with levocarnitine and riboflavin but unfortunately re-hospitalization for evaluation of neurological status and biochemical investigations was denied.

Although we reported a novel mutation in the *L2HGDH* gene, the small sample size was the main limitation of the study which makes genotype-phenotype correlation difficult

Conclusion

Our report further expands the genetic spectrum of L-2-HGA. It also suggests a successful response to treatment with levocarnitine and riboflavin. Whether the response is sustained and correlated to the genotype and WM abnormalities, it needs to be further studied in future reports.

Conflict of interest

The authors declare that there is no conflict of interest.

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Consent for publication

Written informed consent was obtained from the probands' parents for publications of their clinical details.

References

Augoustides-savvopoulou P., Salomons GS., Dotis J., Roilides E., Leontsini M., Jakobs C., and Panteliadis C. (2007). Mutation analysis a prerequisite for prenatal diagnosis of L-2-hydroxyglutaric aciduria?. Molecular Genetics and Metabolism. 91(4). PP 399-401.

Fourati H., Ellouze E., Ahmadia M., Chaari D., Kamoun F., Hsairi I., Triki C., and Mnif Z. (2016). MRI features in 17 patients with l2 hydroxyglutaric aciduria. European Journal of Radiology Open. 27(3). PP 245-250.

Jequier GM., Roulet-Perez E., Meagher-Villemure K., Jacobs C., Salomons GS., Boulat O., Superti-Furga A., Ballhausen D., and Bonafe L. (2008). Sudden unexpected death in an infant with L-2- hydroxyglutaric aciduria. European Journal of Pediatrics. 168(8). PP 957– 962

Kranendijk M., Struys EA., Salomons GS., Van der Knaap MS., and Jakobs C.

(2012). Progress in understanding 2-hydroxyglutaric acidurias. Journal of Inherited Metabolic Disease. 35(4). PP 571–587.

Larnaout A., Amouri R., Kefi M., and Hentati F. (2008). L-2-Hydroxyglutaric aciduria: Clinical and molecular study in three Tunisian families. Identification of a new mutation and inter-familial phenotype variability. Journal of Inherited Metabolic Disease. 31(Suppl 2). S 375–379.

Muthusamy K., Sudhakar SV., Christudass CS., Ghandran M., Thomas M., Gibikote S. (2019). Clinicoradiological Spectrum of L-2-Hydroxy Glutaric Aciduria: Typical and Atypical findings in an Indian Cohort. Journal of Clinical Imaging Science. 9(3). doi:10.25259/JCIS-9-3

Samuraki M., Komai K,. Hasegawa Y., Kimura M., Yamaguchi S., Terada N., and Yamada M. (2008). A successfully treated adult patient with l-2-hydroxyglutaric aciduria. Neurology. 70(13): PP 1051–1052

Sass JO., Jobard F., Topçu M., Mahfoud A., Werle E., Cure S., Al-Sannaa N., Alshawan S.A., Bataillard M., Cimbalistiene L., Grolik C., Kemmerich V., Omran H., Sztriha L., Tabache M., and Fischer J. (2008). $_{L}$-2-Hydroxyglutaric aciduria: Identification of ten novel mutations in the L2HGDH gene. Journal of Inherited Metabolic Disease. 31(Suppl 2). S 275–279.

Steenweg ME., Jakobs C., Errami A., van Dooren SJM., Adeva Bartolome MT., Aerssens P., and Salomons G. (2010). An overview of L-2-hydroxyglutarate dehydrogenase gene (L2HGDH) variants: a genotype-phenotype study. Human Mutatation. 31(4): PP 380–390.

Steenweg ME., Salomons GS., Yapici Z., Uziel G., Scalais E., Zafeiriou DI., Ruiz-

Falco ML., Mejaski-Bosnjak V., Jakobs C., and van der Knaap MS. (2009). L-2-Hydroxyglutaric aciduria: pattern of MR imaging abnormalities in 56 patients. Radiology. 251(3): PP 856–865.

Van Schaftingen E., Rzem R., and Veiga-da-Cunha M. (2009). L-2-Hydroxyglutaric aciduria, a disorder of metabolite repair. Journal of Inherited Metabolic Disease. 32(2): PP 135–142.

Yilmaz K. (2009). Riboflavin treatment in a case with L-2-hydroxyglutaric aciduria. European Journal of Pediatric Neurology. 13(1): PP 57–60.

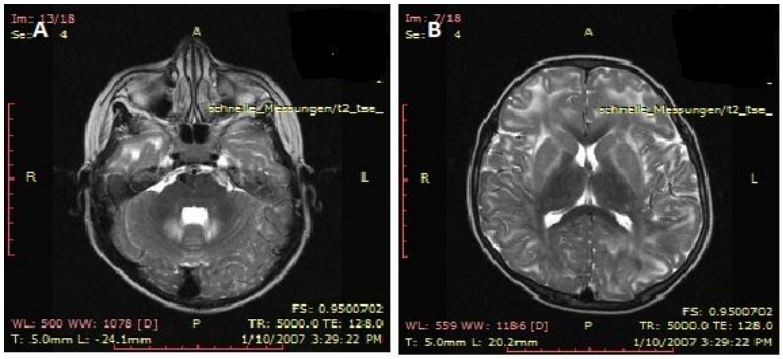

Figure 1: (A) Axial T2-weighted MR images of the brain of patient 1 showing high signal intensity of the dentate nuclei sparing the brainstem and cerebellar white matter, a finding that is characteristic of L2-OH-glutaric aciduria. (B) Bilateral confluent areas of high signal intensity in the subcortical white matter and subcortical U-fibers sparing the deep white matter of the periventricular region, corpus callosum and internal capsule.

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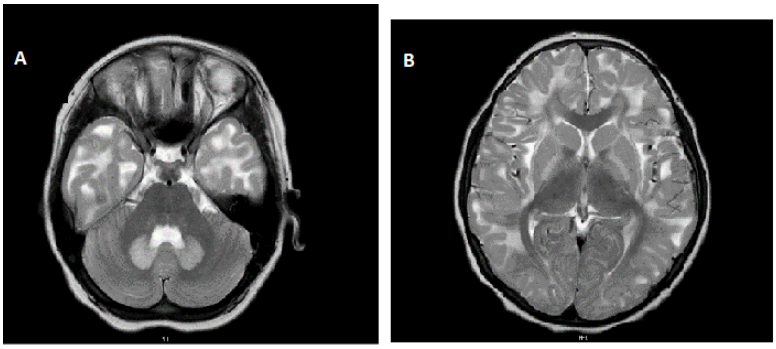

Figure 2. (A) Axial T2-weighted MR images of the brain of patient 2 showing high signal intensity of the dentate nuclei sparing the brain stem and cerebellar white matter. (B) Bilateral confluent areas of high signal intensity in the subcortical white matter, with the involvement of subcortical U-fibers sparing the deep white matter of the periventricular region, corpus callosum and internal capsule.

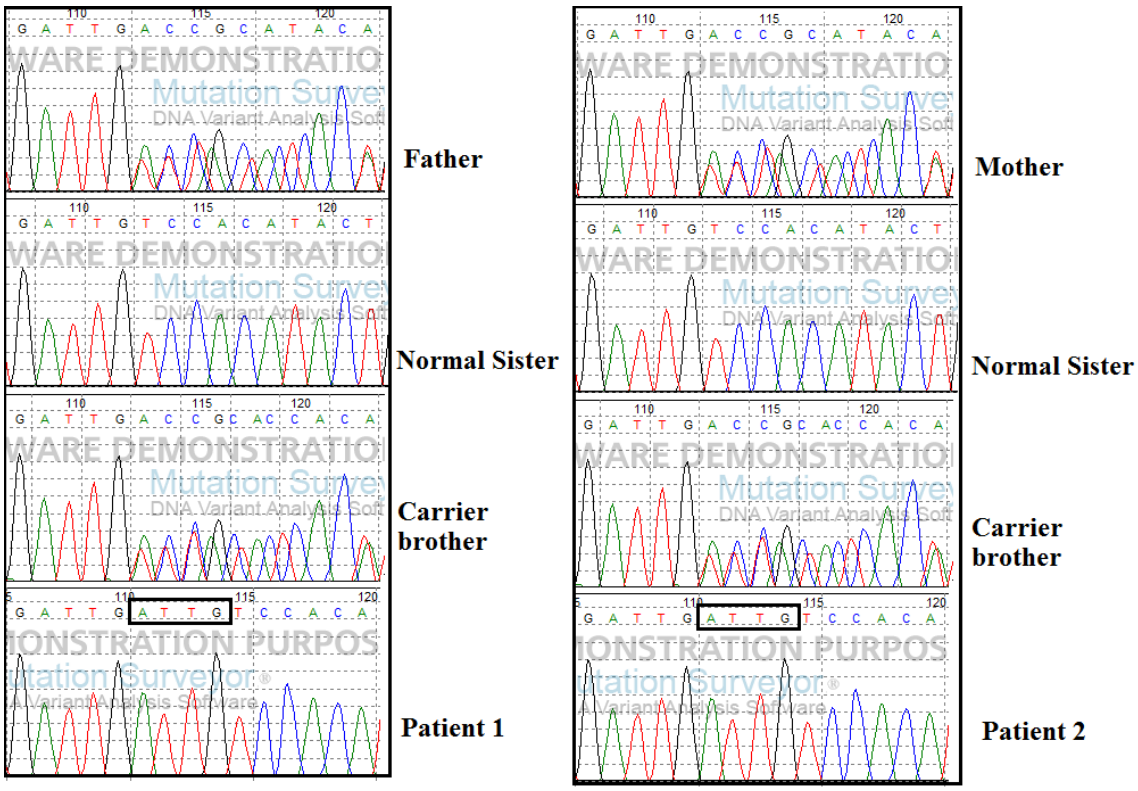

Figure 3. *Mutation analysis*: Direct sequencing and segregation study of L2HGDH (exon5) for the family showed that both patients were homozygous for the novel mutation c.560_565insATTG (p.C187XfsX1), the parents and two healthy brothers were heterozygous for the same mutation while the two healthy sisters were homozygous for the wild allele.

ملخص البحث

حموضة ل-2-هيدروكسيغلوتاريك أسيد هي مرض وراثي نادر من أمراض الأيض التي تصيب الدماغ ناتج عن نقص خميرة ل-2--هيدروكسيغلوتاريت دهيدروجينيز والذي يحول ل-2-هيدروكسيغلوتاريت إلى ألفا-كيتوغلوتاريت. تراكم حامض ل-2-هيدروكسيغلوتاريت سام لخلايا الدماغ ويؤدي إلى ضرر خلايا وأنسجة الدماغ.

لقد سجلنا طفرة جينية لأول مرة لأخوين بهذا المرض ووصفنا الحالة السريرية لهما. علامات المرض تمثلت بتأخر النمو, ترنح ناتج عن خلل في المخيخ, رجفة في اليدين وتراجع مضطرد في النطق. فحص الأحماض العضوية في البول أظهر زيادة في إفراز حامض 2-هيدروكسيغلوتاريت. الرنين المغناطيسي للدماغ أظهر خلل في المادة البضاء تحت قشرة الدماغ و النواة المسننة دون إصابة النواة القاعدية في الدماغ. تحليل الجين المسبب للمرض أظهر إصابتهما بطفرة تسجل للمرة الأولى في القطعة الخامسة من الجين.

المريضان أظهرا استجابة لعلاج الرايبوفلافين والكارنتين وتحسن في الأعراض.

النتيجة: هذا البحث يزيد من عدد الطفرات الجينية المسجلة لهذا المرض كما يشير إلى استجابته للعلاج بدواء الرايبوفلافين والكارنتين